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(57) Abstract

This invention provides isolated nucleic acid molecules encoding an OLD–35 protein, OLD–64 protein, OLD–137 protein, OLD–139 protein, OLD–142 protein or OLD–175 protein. This invention further provides a purified OLD–35 protein, OLD–64, OLD–137, OLD–139, OLD–142 and OLD–175. Finally, this invention provides different uses of the nucleic acids and proteins.

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GENES DISPLAYING ENHANCED EXPRESSION DURING CELLULAR SENESCENCE AND TERMINAL CELL DIFFERENTIATION AND USES THEREOF

This application claims priority and is a continuation-in-part application of U.S. Serial No. 09/243,277, filed February 2, 1999, the contents of which is hereby incorporated by reference.

Throughout this application, various publications are referred to by arabic numeral within parentheses. Full citations for these publications are presented immediately before the claims. Disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

BACKGROUND OF THE INVENTION

Normal cells cultured *in vitro* lose their proliferative potential after a finite number of doublings in a process described as cellular senescence (Hayflick and Moorehead, 1976). This phenomenon is not only well-established in human diploid fibroblasts based on the studies of Hayflick and Moorehead (1976), but it has also been validated by investigations using many additional cell types (Goldstein *et al.*, 1990; Murano *et al.*, 1991). These investigations document an inverse correlation between replicative senescence and donor age and a direct relationship between replicative senescence and donor species lifespan (Hayflick and Moorehead, 1976; Goldstein *et al.*, 1990; Murano *et al.*, 1991). In agreement with this association, cells from patients with premature aging syndromes, such as Werner's syndrome and Progeria, achieve a quiescent state much more rapidly than normal human fibroblasts. In this context, if similar senescence related changes occur in normal fibroblasts, albeit with delayed kinetics, these cell systems represent excellent models for studying senescence *in vitro* and identifying genes relevant to the aging process.

Senescence is characterized by changes in cell morphology, lack of responsiveness to mitogenic stimulation and irreversible growth arrest. However, cells can withdraw from the cell cycle and become non-dividing not only during senescence but also during the processes of DNA damage, apoptosis or terminal differentiation. While senescence is a time-dependent process (Campisi *et al.*, 1995), terminal differentiation can be induced in a variety of cell types by appropriate treatment (Roberts *et al.*, 1999). For example, terminal differentiation can be induced by cAMP treatment in melanocytes (Medrano et al., 1994). Gene expression analysis in terminally differentiated versus senescent melanocytes indicates both similarities and differences (Medrano et al., 1994). Although both pathways result in an elevation in p21 and an inability to phosphorylate ERK2, only the differentiated cells display elevated levels of p27 and the melanocyte-specific transcription factor (MITF) (Medrano et al., 1994; Smith and Pereira-Smith, 1996).

Human melanoma represents an excellent model for studying irreversible growth arrest and terminal differentiation, since these physiological changes can be chemically induced by IFN-β plus mezerein (MEZ) (Fisher et al., 1985; Jiang et al., 1994a). The induction of terminal differentiation in HO-1 human melanoma cells correlates with up-regulation of c-jun, jun-B, α_5 Integrin, β_1 Integrin, fibronectin, HLA Class I, ISG-54, ISG-15 and gro/MSGA as well as down-regulation of c-myc (Jiang *et al.*, 1993a). To define the repertoire of genes differentially expressed during induction of irreversible growth arrest and terminal differentiation in human melanoma cells we have used a rapid and efficient differentiation induction subtraction hybridization (DISH) approach (Jiang and Fisher, 1993). Using this approach alone and in combination with high throughput screening strategies, microchip DNA arrays, a large number of novel genes of potential relevance to growth control and terminal differentiation have been identified and cloned (Jiang et

al., 1995a, 1995b; Lin et al., 1996, 1998; Huang et al., 1999).

On the basis of the considerations described above, it is probable that specific differentially expressed genes may be present within a terminally differentiated cDNA library that also display modified expression during cellular senescence. To begin to identify these overlapping genes, a temporally spaced subtracted differentiation inducer treated H0-1 human melanoma library was screened with RNA isolated from senescent human fibroblasts. Such a screening protocol yielded twenty-eight known and ten novel cDNAs. Subsequent Northern and reverse Northern blotting analyses revealed differential expression of specific cDNAs. Expression of one of these cDNAs, Old-35 was restricted to terminal differentiation and senescence. In this context, this gene may contribute to pathways leading to growth arrest, a defining component of senescence and terminal differentiation.

Interferons (IFNs) comprise a family of related cytokines with diverse including antiviral, antiproliferative, antitumor and immunomodulatory activities (Stark et al., 1998; *Roberts *et al.*, 1999). IFN studies have focused on two main areas; one involving the clinical use of IFN for therapeutic purposes (Gutterman, 1994), the other employing the IFN system as a paradigm to study the mammalian JAK/STAT signaling cascade (Darnell et al., 1994) that leads to IFN-stimulated gene (ISG) activation. To date, the most extensively studied ISGs include RNA-activated protein kinase (PKR), the 2'-5' oligoadenylate synthetase and the MX proteins (Stark et al., 1998, *Der *et al.*, 1998).

Post-transcriptional regulation of mRNA levels is a pivotal control point in gene expression. Early response genes, such as cytokines, lymphokines and proto-oncogenes are regulated by a cis-acting adenylate-uridylate-rich element (ARE) found in the 3' untranslated region (UTR) of the mRNA (Caput et

al., 1986; Shaw and Kamen, 1988; Chen and Shyu, 1995; Myer et al., 1997). Currently, three classes of destabilizing elements have been identified: AUUUA-lacking elements and AUUUA-containing elements grouped into those with scattered AUUUA motifs (such as proto-oncogenes) and those with overlapping AUUUA motifs (such as growth factors) (Chen et al., 1995; Myer et al., 1997). A transfer of 3'UTR containing ARE to 3'UTR of a stable message, such as β-globin, targets this very stable mRNA for rapid degradation (Shaw and Kamen, 1988). In contrast, the removal of an ARE stabilizes an otherwise labile message (*Miller *et al.*, 1984; *Lee *et al.*, 1988).

The present studies describe the cloning and initial characterization of a novel gene, Old-35, from a terminally differentiated human melanoma cDNA library. mRNA stability studies document that Old-35 mRNA, which contains ARE elements, may be stabilized in HO-1 cells by treatment with IFN-β and IFN-b + MEZ. Based on the growth suppressive effect of IFN-β on HO-1 cells, as well as the increased stability of Old-35 during confluence and senescence, it is possible that this gene plays a prominent role in growth suppression induced by this cytokine. Further experimentation is required to define the precise role of Old-35 in IFN signaling, terminal differentiation and cellular senescence. Full-length cloning and subsequent protein analyses should provide insights into the function of this potentially important gene in the processes of aging and differentiation.

Since the processes of terminal differentiation and senescence exhibit strikingly similar characteristics, it is possible that related and overlapping genes and gene expression changes associate with and mediate both of these phenomena. Old-35 was isolated by screening a subtracted human melanoma cDNA library enriched for genes related to growth arrest and terminal differentiation with RNA from senescent human fibroblasts. This cDNA encodes an IFN-β inducible gene expressed during different types of growth

arrest including confluence, senescence and terminal differentiation. Old-35 RNA exhibits increased stability in IFN-β and INF-β + MEZ treated H0-1 human melanoma cells. Steady-state mRNA for Old-35 is also highly expressed in heart and brain, human tissues without active regenerative properties. Judging from the pattern of Old-35 expression, it is possible that this gene may play a prominent role during growth arrest and in this context contributes to the important processes of senescence and terminal differentiation.

SUMMARY OF THE INVENTION

This invention provides isolated nucleic acid molecule encoding an old 35 protein, 64 protein, 137 protein, 139 protein, 142 protein and a 175 protein. The isolated nucleic acid may be a DNA, genomic DNA, cDNA, synthetic DNA or RNA. The isolated nucleic acid has a sequence substantially the same as SEQ ID. Nos. 39, 19, 31, 32, 34 and 38 which are respectively Old 35, old 64, old 137, old 139, old 142 and old 175.

This invention also provides a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding an old 35 protein, 64 protein, 137 protein, 139 protein, 142 protein and a 175 protein. The nucleic acid probe may be DNA, genomic DNA, cDNA, synthetic DNA or RNA.

This invention further provides a host vector system for the production of a protein having the biological activity of old 35, 64, 137, 139, 142 and 175. The isolated old 35, 64, 137, 139, 142 and 175 nucleic acid molecule is linked to a promoter of RNA transcription and then to a plasmid. The suitable host is a bacterial cell, insect cell, or animal cell, depending on the type of promoter and plasmid used. This invention also provides a method of producing a protein having the biological activity of old 35, 64, 137, 139, 142 and 175, which comprises growing the selected host vector system under suitable conditions permitting production of the protein and recovering the protein so produced.

This invention further provides purified protein of old 35, 64, 137, 139, 142 and 175. Such purified old 35, 64, 137, 139, 142 and 175 will be useful for inhibiting growth of cancer cells. This invention provides a method of contacting the cancer cells with an amount of old 35, 64, 137, 139, 142 and 175 at a concentration effective to inhibit growth of cancer cells. This invention further provides a method of determining whether a cell is senescent by (a)isolating the

nucleic acids in the cell (b) hybridizing the isolated nucleic acids with the nucleic acid of old 35 or 64 under conditions permitting hybrids formation and (c) detecting the expression of old 35 or old 64 in the cell. This invention further provides a method of determining whether a cell has growth arrest by (a) isolating the nucleic acids in the cell; (b) hybridizing the isolated nucleic acids with the nucleic acid of old 35 or 64 under conditions permitting hybrids formation; and (c) detecting the expression of old 35 or old 64 in the cell. This invention further provides a method of determining whether a cell has terminal differentiation by (a) isolating the nucleic acids in the cell; (b) hybridizing the isolated nucleic acids with the nucleic acid of old 35 or 64 under conditions permitting hybrids formation; and (c) detecting the expression of old 35 or old 64 in the cell. Further, this invention provides that the detector used is a DNA, RNA or protein. This invention also provides a method of regenerating tissue with an inhibitor of old 35 protein at a concentration effective to regenerate said tissues. This invention provides a method of anti-aging in a cell comprising contacting the cell with an agent for inhibiting expression of old 35 at a concentration effective to reverse growth arrest in the cell. Finally, this invention provides a pharmaceutical composition for stimulating cell growth comprising a pharmaceutically acceptable carrier and purified old 35 or old 64 at a concentration effective to stimulate cell growth.

BRIEF DESCRIPTION OF FIGURES

Figure 1 Expression of Old-35 in H0-1 human melanoma cells treated with IFN-β or FN-β + MEZ, young human fibroblasts and two different types of senescent Progeria human fibroblasts. Northern blot contains 10μg of total RNA from control untreated HO-1 (lane 1), IFN-β treated (2,000 U/ml) H0-1 (lane 2), IFN-β + MEZ treated (2,00U/ml + 10ng/ml) H0-1 (lane 3), young fibroblasts (GM01379) (lane 4), and two senescent Progeria cell lines (AG01976) (lane 5) (AG0989B) (lane 6). Blots were exposed for autoradiography for 1, 4 or 24 hr. EtBr staining for quantification of gel loading and determining RNA quality.

Figure 2 Effect of IFN-α, IFN-β, IFN-γ, TNF-α and IFN-β + MEZ on Old-35 expression in H0-1 cells. All Northern blots contain 10 mg of total RNA. (A) Time course induction of Old-35 by IFN-β in H0-1 cells. Cells were seeded at ~60% confluence and treated with IFN-β (2,000 units/ml) and RNA was isolated at the indicated time. U = RNA from control, untreated cells. (B) Dose response expression of Old-35 in H0-1 cells treated with IFN-β (2,000 units/ml). RNAs were isolated after 24 hr treatment. (C) Effect of IFN-α (Iα), IFN-β (Iβ), IFN-γ (Iγ) and TNF-α (Tα) on Old-35 expression in H0-1 cells. RNAs were isolated after 15 hr treatment with 1,000 units/ml of the different agents. U = RNA from control, untreated cells. (D) Time course induction of Old-35 by IFN-β + MEZ in H0-1 cells. RNAs were isolated from cells treated with 2,000 units/ml of IFN-β + 10 ng/ml of MEZ.

Figure 3 Expression of Old-35 in various human tissues and during mouse development. (A) Northern blot

contains 2μg of poly A^+ RNA per lane from eight different human tissues. Lanes 1-8 contain, in order, RNA from human heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas (Clontech). (B) Northern blot contains 10μg of total RNA from mouse embryos. The number of days signifies days post-gestation.

Figure 4 Sequence comparison between human and the mouse homologue of Old-35. Upper panel sequence of human Old-35 (h-Old-35); Middle panel: sequence of mouse Old-35 (m-Old-35); and Lower panel: shared consensus sequences between human and mouse Old-35.

Figure 5 Expression of Old-35 in IDH4 cells grown in the presence or absence of Dex. Northern blot contains 10μg of total RNA per lane from IDH-4 cells. + Dex = cells grown continuously in the presence of 10^{-6} M Dex; - Dex = cells grown for the indicated days in the absence of Dex. For the latter experiment, cells were grown in the presence of Dex and then shifted to charcoal stripped media and grown for 3, 5, 7 and 14 days without Dex.

Figure 6 Expression of Old-35 and p21 during cell cycle progression in human skin fibroblasts. Northern blot contains 10μg of total RNA per lane from normal human fibroblasts. Confluent normal fibroblasts (C) were trypsinized and reseeded (1:2). Total RNA was collected at 5, 15 and 20 hr after reseeding. At 20hr following subculture, the cells were 90% confluent.

Figure 7 AU rich sequences found in the 3' untranslated region (UTR) of several lymphokine and

protooncogene mRNAs. Abbreviations: Abbreviations: Hu-human, GM-CSF = granulocyte-monocyte colony stimulating factor; IFN-α = interferon α; IL 2 = Interleukin 2; TNF = tumor necrosis factor; c-fos = fos proto-oncogene. The underlined/overlined AUUUA motif if the largest sequence common to all mRNAs shown. References: HuGM-CSF (Wong et al., 1985), HuIFN-α (Goeddel et al., 1983), Hu IL 2 (Kashima et al., 1985), HuTNF (Nedwin et al., 1985), Hu c-fos (van Straaten et al., 1983).

Figure 8 Effect of cycloheximide treatment on Old-35 expression in H0-1 cells and the half-life of Old-35 mRNA in IFN-β + MEZ treated H0-1 cells. Each lane in the Northern blots contains 10μg of total RNA. (A) HO-1 cells were pre-treated with cyclohexamide 50mg/ml for 30 min and then treated with IFN-β for 2, 3 or 4 hr (**lanes 2, 3, and 4**, respectively). HO-1 cells were pre-treated with IFN-β for 5 hr (**lane 5**) and then treated with cycloheximide for 15 hr (**lane 6**). U = RNA from control untreated HO-1 cells. (B) Half-life of Old-35 mRNA in IFN-β + MEZ (IM) (2,000 units/ml + 10 ng/ml) treated H0-1 cells. Cells were incubated with IM for 15 hr and then exposed to ActD (50 mg/ml) for 2, 6, 8, 10 and 12 hr. U = RNA from control untreated H0-1 cells. AD = RNA from control H0-1 cells treated with ActD (5 μg/ml).

Figure 9 DNA sequence and predicted encoded protein of Old-35. (A) cDNA sequence of Old-35. Alternate polyadenylation site is underlined. This site is present in 10% of all cDNAs (*Manley *et al.*, 1988). (B) Predicted protein encoded by the Old-35 cDNA.

Figure 10 Sequence similarity between the bacterial protein PNPase and the predicted protein sequence of Old-35. Upper Panel: *Bacillus subtilis* PNPase sequence. Middle Panel: predicted human Old-35 protein sequence. Lower Panel: regions of consensus amino acids between the bacterial PNPase protein sequence and the predicted Old-35 protein sequence. Black boxed areas indicates amino acid identity and gray boxed areas indicate amino acid similarities between the bacterial PNPase and the predicted Old-35 encoded protein.

Figure 11 Northern Blot of HO-1, confluent HO-1, IFN-β treated, IFN-β+MEZ treated HO-1 treated with Actinomycin D (50mg/ml). Total RNAs were collected 2,4,6,8,10,12 after the AD treatment. Old-35 cDNA was used as a probe. Ethydium Bromide was shown for loading control

Figure 12 Northern Blot of IDH4 and AR5 cells. IDH4 cells contain dexamethasone (DEX) inducible mouse mammary tumor virus-driven simian virus 40 T-antigen. Total RNA was extracted from cells treated with DEX (indicated as +), and from cells growing without DEX for 3,5,7, and 14 days). AR5 cells contain temperature sensitive simian virus 40 T-antigen. Total RNA was collected from cells at 35C and 1,3,7,14 days after shift to 39C. Old-35 and p21 were used as a probe.

Figure 13 Structure of Old-35 gene. RnasePH, KH, S1 signify domains found in Old-35 cDNA. Top picture shows two different versions of Old-35 which vary in the 3'UTR length
The bottom picture shows cloning of the Old-35 cDNA using C-ORF technique.

Figure 14

Localization of GFP-Old-35, and GFP alone in HeLa cells.

Figure 15

In situ hybridization to mouse embryo (9.5 days) using murine Old-35. The arrows indicate the expression in the spinal column.

Figure 16

Northern blot of HO-1 cells treated with different subtypes of IFN-α using Old-35 as a probe. IFN-β was used as a control.

DETAILED DESCRIPTION OF THE INVENTION

In order to facilitate an understanding of the Experimental Details section which follows, certain frequently occurring methods and/or terms are described in Sambrook, et al. (1989).

Throughout this application, the following standard abbreviations are used throughout the specification to indicate specific nucleotides:

C=cytosine	A=adenosine
T=thymidine	G=guanosine

This invention provides an isolated nucleic acid molecule encoding an OLD-35 or OLD-64 protein. In an embodiment, the above nucleic acid molecule comprises a nucleic acid having a sequence substantially the same as set forth in SEQ. ID. No.39 or 19.

This invention also provides isolated nucleic acid molecules encoding an OLD-137, OLD-139, OLD-142, or OLD-175 protein. In an embodiment, the nucleic acid comprises a nucleic acid having a sequence substantially the same as set forth in SEQ. ID. Nos.31, 32, 34 or 38. The above-described nucleic acid may be DNA, genomic DNA, cDNA, synthetic DNA, or RNA.

This invention also encompasses nucleic acid which encode amino acid sequences which differ from those of OLD-35, OLD-64, OLD-137, OLD-139, OLD-142 or OLD-175, but which should not produce phenotypic changes. Alternatively, this invention also encompasses DNAs and cDNAs which hybridize to the DNA and cDNA of the subject invention. Hybridization methods are well known to those of skill in the art.

The DNA molecules of the subject invention also include DNA molecules coding for protein analogs, fragments or derivatives of antigenic proteins which differ from naturally-occurring forms in terms of the identity or location of one or more amino acid residues (deletion analogs

containing less than all of the residues specified for the protein, substitution analogs wherein one or more residues specified are replaced by other residues and addition analogs wherein one or more amino acid residues is added to a terminal or medial portion of the proteins) and which share some or all properties of naturally-occurring forms. These sequences include: the incorporation of codons "preferred" for expression by selected non-mammalian host; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate DNA sequences that facilitate construction of readily expressed vectors.

The nucleic acid molecule described and claimed herein are useful for the information which they provide concerning the amino acid sequence of the protein and as products for the large scale synthesis of the protein by a variety of recombinant techniques. The molecule is useful for generating new cloning and expression vectors, transformed and transfected procaryotic and eukaryotic host cells, and new and useful methods for cultured growth of such host cells capable of expression of the protein and related products.

The invention also provides fragments or portion of the Old gene or protein wherein the biological activity of said gene product is maintained. Such fragment or portion may join to other amino acid sequence to create a multi-functional molecule. It is within the ordinary skill to determine such biologically active fragment or portion. A trimming experiment may be performed to define said fragment of portion.

Old-35, Old-64, Old-137, Old-139, Old-142 or Old-175 may be isolated in a variety of vertebrates. In an embodiment, a human Old-35, Old-64, Old-137, Old-139, Old-142 and Old-175 are isolated.

The isolated nucleic molecule of Old-35, Old-64, Old-137,

Old-139, Old-142 and Old-175 are represented respectively by SEQ. ID. Nos. 39, 19, 31, 32, 34 and 38.

This invention provides a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a Old-35, Old-64, Old-137, Old-139, Old-142 or Old-175. In an embodiment, the nucleic acid is DNA, genomic DNA, cDNA, synthetic DNA or RNA.

As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs. The nucleic acid molecule will be specific to said Old genes i.e. under appropriate conditions, the molecule will only hybridize with said old gene and no other genes. Said molecule may contain an unique sequence of said Old gene.

Nucleic acid probe technology is well known to those skilled in the art who will readily appreciate that such probes may vary greatly in length and may be labeled with a detectable label, such as a radioisotope or fluorescent dye, to facilitate detection of the probe.

Probe molecules may be produced by insertion of a nucleic acid molecule which encodes OLD-35, OLD-64, OLD-137, OLD-139, OLD-142 or OLD-175 protein or a fragment thereof into suitable vectors, such as plasmids or bacteriophages, followed by transforming into suitable bacterial host cells, replication in the transformed bacterial host cells and harvesting of the DNA probes, using methods well known in the art. Alternatively, probes may be generated chemically from DNA synthesizers.

The probes are useful for 'in situ' hybridization to locate tissues which express this gene, or for other hybridization

assays for the presence of this gene or its mRNA in various biological tissues.

The invention also provides an antisense nucleic acid molecule comprising a sequence complementary to the nucleic acid which encodes OLD-35, OLD-64, OLD-137, OLD-139, OLD-142 or OLD-175 protein or a fragment thereof. In an embodiment, the antisense nucleic acid molecule is capable of inhibiting the expression of the hybridized gene.

This invention also provides the above-described isolated nucleic acid molecule operatively linked to a promoter of RNA transcription. This invention further provides a vector which comprises the above-described isolated nucleic acid molecule.

Vectors which comprise the isolated nucleic acid molecule described hereinabove also are provided. Suitable vectors comprise, but are not limited to, a plasmid or a virus. These vectors may be transformed into a suitable host cell to form a host cell vector system for the production of a protein having the biological activity of OLD-35, OLD-64, OLD-137, OLD-139, OLD-142 or OLD-175 protein or a fragment thereof.

This invention further provides an isolated DNA, genomic DNA , cDNA, synthetic DNA or RNA molecule described hereinabove wherein the host cell is selected from the group consisting of bacterial cells (such as E.coli), yeast cells, fungal cells, insect cells and animal cells. Suitable animal cells include, but are not limited to Vero cells, HeLa cells, Cos cells, CV1 cells and various primary mammalian cells.

This invention provides a purified, OLD-35 protein, a purified, OLD-64 protein, a purified, OLD-137 protein, a purified, OLD-139 protein, a purified, OLD-142 protein, and a purified, OLD-175 protein.

This invention also provides a protein encoded by the above-described isolated nucleic acid molecule.

This invention also provides an antibody or antigen-binding fragment thereof that specifically binds to OLD-35, OLD-64, OLD-137, OLD-139, OLD-142 or OLD-175 protein. In an embodiment, the antibody is a monoclonal antibody.

Polyclonal antibodies against these proteins may be produced by immunizing animals using selected peptides determined from the decoded amino acid sequences. Monoclonal antibodies are prepared using hybridoma technology by fusing antibody producing B cells from immunized animals with myeloma cells and selecting the resulting hybridoma cell line producing the desired antibody. Alternatively, monoclonal antibodies may be produced by in vitro techniques known to a person of ordinary skill in the art. These antibodies are useful to detect the expression of the OLD proteins in living animals, in humans, or in biological tissues or fluids isolated from animals or humans.

This invention provides a method of inhibiting growth of cancer cells comprising contacting the cancer cells with an amount of purified OLD-35, OLD-64 protein or a portion thereof effective to inhibit growth of cancer cells.

This invention also provides a method for reversing the cancerous phenotype of a cancer cell which comprises introducing a nucleic acid comprising an Old-35 or Old-64 gene or a portion thereof into the cell under conditions permitting the expression of the gene so as to thereby reverse the cancerous phenotype of the cell.

This invention provides a method for reversing the cancerous phenotype of a cancer cell in a subject which comprises introducing a nucleic acid molecule comprising an Old-35 or Old-64 gene or a portion thereof into the subject's cancerous cell under conditions permitting expression of the gene in

the subject's cell so as to thereby reverse the cancerous phenotype of the cell.

In an embodiment of the method, the nucleic acid molecule comprises a vector. In a further embodiment, the Old-35 or Old-64 gene is linked to a regulatory element such that its expression is under the control of the regulatory element. In a still further embodiment, the regulatory element is a tissue specific regulatory element. In a still further embodiment, the regulatory element is inducible or constitutive. Inducible regulatory element like an inducible promoter is known in the art. Regulatory element such as promoter which can direct constitutive expression is also known in the art.

In a separate embodiment, the regulatory element is a tissue specific regulatory element. The expression of the inserted gene will then be tissue-specific.

Methods to introduce a nucleic acid molecule into cells have been well known in the art. Naked nucleic acid molecule may be introduced into the cell by direct transformation. Alternatively, the nucleic acid molecule may be embedded in liposomes. Accordingly, this invention provides the above methods wherein the nucleic acid is introduced into the cells by naked DNA technology, adenovirus vector, adeno-associated virus vector, Epstein-Barr virus vector, Herpes virus vector, attenuated HIV vector, retroviral vectors, vaccinia virus vector, liposomes, antibody-coated liposomes, mechanical or electrical means. The above recited methods are merely served as examples for feasible means of introduction of the nucleic acid into cells. Other methods known may be also be used in this invention.

This invention provides a method for reversing the cancerous phenotype of a cancer cell which comprises introducing OLD-35 or OLD-64 protein or a portion thereof into the cancerous cell so as to thereby reverse the cancerous phenotype of the

cell.

This invention provides a method for reversing the cancerous phenotype of a cancer cell in a subject which comprises introducing OLD-35 or OLD-64 protein into the subject's cancerous cell so as to thereby reverse the cancerous phenotype of the cell. In an embodiment, the cancer cell is a breast, cervical, colon, pancreatic, thyroid, skin, brain, prostate, nasopharyngeal, lung, glioblastoma multiforme, lymphoma, leukemia, connective tissue, nervous system cell or basal cell.

This invention further provides a pharmaceutical composition which comprises an amount of a nucleic acid molecule comprising Old-35, Old-64 gene or portion thereof effective to reverse the cancerous phenotype of a cancer cell and a pharmaceutically acceptable carrier. In an embodiment, the nucleic acid molecule comprises a vector. In a further embodiment, the vector is an adenovirus vector, adeno-associated virus vector, Epstein-Barr virus vector, Herpes virus vector, attenuated HIV vector, retrovirus vector or vaccinia virus vector.

This invention also provides a pharmaceutical composition comprising an amount of OLD-35 or OLD-64 protein effective to reverse the cancerous phenotype of a cancer cell and a pharmaceutically acceptable carrier. In an embodiment, the cancer cell is a breast, cervical, colon, pancreatic, thyroid, skin, brain, prostate, nasopharyngeal, lung, glioblastoma multiforme, lymphoma, leukemia, connective tissue, nervous system or basal cell.

In an embodiment of the above methods, the nucleic acid comprises a vector. The vector includes, but is not limited to, an adenovirus vector, adeno-associated virus vector, Epstein-Barr virus vector, Herpes virus vector, attenuated HIV vector, retrovirus vector and vaccinia virus vector.

As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers. The pharmaceutical composition may be constituted into any form suitable for the mode of administration selected. Compositions suitable for oral administration include solid forms, such as pills, capsules, granules, tablets, and powders, and liquid forms, such as solutions, syrups, elixirs, and suspensions. Forms useful for parenteral administration include sterile solutions, emulsions, and suspensions.

In the practice of the method administration may comprise daily, weekly, monthly, hourly or by peak and trough, the precise frequency being subject to various variables such as age and condition of the subject, amount to be administered, half-life of the agent in the subject, area of the subject to which administration is desired and the like.

In connection with the method of this invention, a therapeutically effective amount may include dosages which take into account the size and weight of the subject, the age of the subject, the severity of the symptom, the efficacy of the agent and the method of delivery of the agent. One of ordinary skill in the art would be readily able to determine the exact dosages and exact times of administration based upon such factors.

This invention provides a method of determining whether a cell is senescent comprising measurement of the expression of Old-35 gene, wherein the expression of the Old-35 gene indicates that the cell is senescent. In an embodiment, the expression of the Old-35 gene is measured by the expression of Old-35 specific RNA. In another embodiment, the expression of the Old-35 gene is measured by the expression of the OLD-35 protein.

This invention also provides a method of determining whether a cell is terminally differentiated comprising measurement

of the expression of Old-35 gene, wherein the expression of the Old-35 gene indicates that the cell is terminally differentiated. In an embodiment, the expression of Old- 35 gene is measured by the expression of Old-35 specific RNA. In another embodiment, the expression of the Old-35 is measured by the expression of OLD-35 protein.

This invention provides a method of determining whether a cell has growth arrest comprising measurement of the expression of Old-35 gene, wherein the expression of Old-35 gene indicates that the cell has growth arrest. In an embodiment, the expression of the Old-35 gene is measured by the expression of old 35 specific RNA. In another embodiment, the expression of the Old-35 gene is measured by the expression of OLD-35 protein.

This invention provides a method of inhibiting growth of cancer cells comprising contacting the cancer cells with an amount of purified OLD-64 protein effective to inhibit growth of cancer cells.

This invention also provides a method of inhibiting growth of cancer cells comprising contacting the cancer cells with an amount of purified OLD-64 protein effective to inhibit growth of cancer cells.

This invention provides a method of determining whether a cell is senescent comprising measurement of the expression of Old-64 gene, wherein the expression of the Old-64 gene indicates that the cell is senescent. In an embodiment, the expression of old-64 gene is measured by the expression of Old-64 specific RNA. In another embodiment, the expression of Old-64 gene is measured by the expression of the OLD-64 protein.

The expression of specific OLD RNA may be measured by the below method: (a) isolating the nucleic acids from a sample; (b) hybridizing the isolated nucleic acids with the

appropriate Old gene under conditions permitting hybrids formation; and (c) detecting the hybrid formed.

The invention provides a pharmaceutical composition for inhibiting cell growth comprising a pharmaceutically acceptable carrier and purified old 35 or old 64 at a concentration effective to inhibit cell growth.

This invention provides a method of determining whether a cell is terminally differentiated comprising measurement of the expression of Old-64 gene, wherein the expression of the Old-64 gene indicates that the cell is terminally differentiated. In an embodiment, the expression of Old-64 gene is measured by the expression of Old-64 specific RNA. In another embodiment,the expression of Old- 64 gene is measured by the expression of the OLD-64 protein.

This invention provides a method of determining whether a cell is growth arrested comprising measurement of the expression of Old-64 gene, wherein the expression of Old-64 gene indicates that the cell is growth arrested. In an embodiment, the expression of Old- 64 gene is measured by the expression of Old-64 specific RNA. In another embodiment, the expression of Old- 64 gene is measured by the expression of the OLD-64 protein.

This invention provides a method of regenerating tissues comprising contacting the tissue with an inhibitor of OLD-35 or OLD-64 protein at a concentration effective to regenerate said tissues.

Methods to determine such a concentration are well-known in the art. The effective concentration of said inhibitor of OLD-35 or OLD-64 protein may be determined by using different concentrations of said inhibitor and examine the effect produced.

This invention provides a method of anti-aging in a cell

comprising contacting the cell with an agent for inhibiting expression of Old-35 or Old-64 gene at a concentration effective to reverse the aging process in the cell.

This invention provides a pharmaceutical composition for stimulating or resuming cell growth comprising a pharmaceutically acceptable carrier and purified Old-35 or Old-64 suppressant at a concentration effective to stimulate or resuming cell growth. A purified suppressant is compound capable of suppressing the activity of OLD-35 or OLD-64. For example, the suppressant can act on the gene level such that no Old-35 or Old-64 gene will be switched on. Alternatively, the suppressant may be a samll molecule capable of binding to the active sites on the OLD-35 or -64 protein such that the protein will not be functional or the activity of the protein will decrease. A specific antibody or its binding fragment, which is capable of binding to the OLD-35 or -64, may be a suppressant.

This invention provides a method for screening the presence of interferon alpha or beta of a sample comprising steps of:(a) contacting the sample with cells under conditions permitting expression of Old-35 or Old-64 gene in the presence of interferon alpha or beta; and (b) determining the expression of Old-35 or Old-64 gene, an increase of expression indicates the presence of interferon alpha or beta.

This invention provides a method for detection of the secretion of interferon alpha or beta comprising steps of: (a) obtaining an appropriate sample from the subject; and (b) detecting expression of Old-35 or Old-64 gene, the expression of Old-35 or Old-64 gene indicating the secretion of interferon in a subject.

This invention provides a method for monitoring chemotherapy of a subject comprising steps of: (a) obtaining an

appropriate sample from the subject; and (b) detecting expression of Old-35 or Old-64 gene, the expression of Old-35 or Old-64 gene indicating that the chemotherapy is effective.

This invention provides a method for diagnosis of the proliferating stage of a tumor from a subject comprising steps of:(a) obtaining an appropriate sample from the subject; and (b) detecting expression of Old-35 or Old-64 gene, the expression of Old-35 or Old-64 gene indicating that the tumor is not at a proliferating stage.

This invention also provides a kit for diagnosis of the proliferating stage of a tumor, comprising a nucleic acid molecule capable of specifically hybridizing to the nucleic acid molecule of Old-35 or Old-64.

This invention also provides a kit for diagnosis of the proliferating stage of a tumor, comprising antibody capable of specifically recognizing OLD-35 or OLD-64 protein.

This invention provides different kits containing appropriate reagents to perform the above-described methods.

This invention also provides a method for identifying an agent that modulates the expression of Old-35 or Old-64 gene, comprising: (a)contacting a candidate agent with a cell transformed or transfected with a reporter gene under the control of a Old-35 or Old-64 promoter or a regulatory element thereof under conditions and for a time sufficient to allow the candidate agent to directly or indirectly alter expression of the promoter or regulatory element thereof; and (b) determining the effect of the candidate agent on the level of reporter protein produced by the cell, thereby identifying an agent that modulates expression of Old-35 or 64 gene.

This invention provides a method of identifying compounds that induce proliferation or cancerous phenotype,

comprising: exposing cell comprising the promoter of Old-35 or Old-64 to the compound and identifying compounds that suppress the Old-35 or 64 promoter.

This invention provides a method of identifying compounds that induces senescence, or terminal differentiation, comprising: exposing the cell comprising the promoter of Old-35 or Old-64 to the compound and identifying compounds that activate the Old-35 or 64 promoter.

This invention provides a method of identifying genes which are common to the pathway of senescence and terminal differentiation comprising steps of: (a) obtaining a subtracted library which is enriched for genes expressed in terminal differentiation; (b) screening the library with senescent probe to identify novel genes which are expressed during senescence and terminal differentiation; and (c) examining the biological activity of the identified gene to determined whether it is expressed during senescence and terminal differentiation.

This invention provides a method of identifying genes which are common to the pathway of senescence and terminal differentiation comprising steps of: (a) obtaining a subtracted library which is enriched for genes expressed in senescence; (b) screening the library with terminal differentiation probe to identify novel genes which are expressed during senescence and terminal differentiation; and (c) examining the biological activity of the identified gene to determined whether it is expressed during senescence and terminal differentiation.

This invention also provides the gene identified by the above methods.

This invention provides a method of degrading specific RNAs in a cell comprising induction of the expression of Old-35. This invention further provides a method of degrading

specific RNAs in a cell comprising introducing a vector into the cell comprising the Old-35 gene.

In one embodiment of the invention, expression of Old-35 can be used as diagnostic indicator of cellular senescence, terminal differentiation and/or growth suppression. Specifically, Old-35 can be used to determine if a cell has lost proliferative ability and thus has become senescent.

In addition, expression of Old-35 can be used as a marker to identify drugs or small molecules that will induce senescence, e.g., to inhibit cancer cell growth or abnormal proliferative states such as psoriasis, hemangioblastoma, etc..

Further, expression of Old-35 can be used to identify drugs or small molecules that will inhibit senescence, and thus stimulate tissue regrowth, repair and/or regeneration.

Still further, expression of Old-35 can be used as a marker to identify drugs or small molecules that will induce terminal cell differentiation, e.g., to inhibit cancer cell growth or abnormal proliferative states such as psoriasis, hemangioblastoma, etc..

Expression of Old-35 can also be used to identify drugs or small molecules that will inhibit terminal differentiation, and thus stimulate tissue regrowth, repair and/or regeneration.

Furthermore, expression of Old-35 can be used as marker for detecting cytokines, specifically type I interferons, in biological samples. Since type I interferons, including leukocyte and fibroblast interferons, which activate gene expression through the well characterized Jak and Stat kinase pathways, this gene (Old-35) can be used to detect or monitor drugs and other small molecules that activate these important pathways.

The combination of Old-35 with other interacting proteins can be used to target the differentiation of specific target cells, and thus result in the reprogramming of pluripotent stem cells to terminally differentiated end cells.

Additionally, Old-35 can be used to selectively stabilize specific mRNAs possibly containing AU rich 3' UTRs (untranslated regions). This effect can result in the sustained expression of genes potentiating or inhibiting cell growth. It could also result in the stabilizing of cytokine genes resulting in increased biological and immunological activity.

Old-35 can also be used as part of a methodology to polymerize random NTPs into nucleic acids and/or to induce the degradation of specific mRNAs.

This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

EXPERIMENTAL DETAILS

Library Screening

A subtracted cDNA library enriched in genes modified during terminal differentiation in human melanoma cells (Jiang and Fisher, 1993) was plated at 200 pfu/plate. Colonies were transferred to Nylon filters, denatured for 2 min (1.5M NaCl, 0.5M NaOH), neutralized for 5 min (1.5M NaCl, 0.5M Tris-HCl, pH 8.0), and washed for 30 sec (0.2M Tris-HCl, pH 7.5, 2 X SSC). Filters were cross-linked (120,000 μJ of UV energy) for 30 sec in a Strata linker (Stratagene) and prehybridized at 65°C for 2 hr in ExpressHyb (ClonTech). The probe was denatured at 95°C for 5 min, cooled at 0°C for 5 min and then applied to the filters at 3 X 10^6 cpm/ml. The filters were hybridized overnight at 65°C. The next day, the filters were washed (2 X SSC, 0.1 % SDS) 3 X for 20 min and exposed for autoradiography.

PREPARATION OF THE PROGERIA CDNA PROBE

Ten μg of total RNA derived from AG0989B cells (Progeria) (p 22) (Corriel Repository, Camden) was reverse transcribed using SuperScript II (manufacturer's protocol, GibcoBRL) except that 900 μCi of [α-^{32}P]-dCTP (3000Ci/mmole) (Amersham) and 0.4mM of non-radioactive dCTP was used in place of 10mM dCTP. The probe was purified using Quick Spin Columns (Boehringer Mannheim).

PHAGE ISOLATION

The exposed film from autoradiography was aligned with the phage containing plates and hybridizing clones were isolated and re-suspended in SM buffer (1 ml).

PCR

PCR was performed for each phage stock using the manufacturer's protocol (GibcoBRL) with 3 μl of SM stock. Since T3 and T7 primers flank the insert, these primers were used to selectively amplify the insert from the phage vector

(Stratagene). PCR conditions were 30 cycles at 94°C for 1 min, 55°C for 1 min, 72°C for 2 min and 72°C for 10 min to allow complete extension. The PCR products were resolved on 1% agarose gels to determine the size of the product. All clones were sequenced and the novel cDNAs were selected for Northern blotting analysis.

NORTHERN BLOTTING

Total RNA was extracted using the guanidinium isothiocyanate method followed by phenol/chloroform/isoamyl extraction and precipitation in isopropanol as described in Chomczynski and Sacchi (1987). The probes were labeled with [α-^{32}P]dCTP by random priming (Amersham). Ten μg of total RNA were electrophoresed in a 1% agarose/2.2M formaldehyde gel and transferred to Hybond-NX filters (Amersham). Hybridization was performed in ExpressHyb solution (Manufacturer's protocol, Clontech). Briefly, filters were prehybridized at 67°C for 0.5 hr, hybridized with a denatured probe for 1.5 hr, and washed (.2 X SSC, .1 % SDS) 1 X at 24°C for 5 min, and 2 X at 55°C for 20 min.

CELLS AND CULTURE CONDITIONS

HO-1 human melanoma cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum at 37°C in a 5% CO_2/95% air humidified incubator. Cell lines used for the senescence study were obtained from Corriel Repository (Camden, NJ). Fibroblast cell lines from patients with Progeria-Hutchinson-Gilford Syndrome (AG01972B, AG0989B, AG01178B) and normal fetal fibroblasts (GM01379A) were grown in DMEM supplemented with 15% fetal bovine serum (Gibco BRL) and 2 X essential and non-essential amino acids (Sigma). IDH4 cells (Wright *et al.*, 1989) were grown in DMEM supplemented with 10% fetal bovine serum or 10% charcoal stripped fetal bovine serum. HO-1 cells were treated with IFN-β (2,000 U/ml) and MEZ (10 ng/ml) to induce terminal differentiation (Fisher et al., 1985). To inhibit RNA and protein synthesis, HO-1 cells were treated with actinomycin D (5 μg/ml) and

cycloheximide (50 μg/ml), respectively, as previously described (Jiang et al., 1993b).

STAINING FOR SENESCENCE-ASSOCIATED (SA) B-GAL ACTIVITY

Cells were washed 2 X with PBS, fixed in 3% formaldehyde, and stained as previously described (Dimri *et al.*, 1995). Briefly, following fixation, cells were incubated overnight at 37°C in a reaction buffer containing X-gal (1 mg/ml), 40mM citric acid/sodium phosphate (pH 6.0), potassium ferrocyanide/ferricyanide (5mM), NaCl (150mM) and 2mM $MgCl_2$. IDH4 cells grown in the presence of dexamethasone (10^{-6} M) were used as a negative control.

EXPERIMENTAL RESULTS

Preliminary screening of cDNA libraries screening the temporally spaced subtracted differentiation inducer treated H0-1 cDNA (DISH) library enriched for genes regulated during terminal differentiation in melanoma cells, with the RNA from senescent fibroblasts, resulted in the identification of 10 novel and 28 known cDNAs, referred to as Old cDNAs (Table 1). Northern and reverse Northern blotting was used to determine the expression patterns of these Old cDNAs. The goal of our screening was to identify and clone differentially expressed genes common to senescence and terminal differentiation. To achieve this aim, RNAs from HO-1 (untreated or treated with IFN-β, 2,000 U/ml or IFN-β (2,000 U/ml) +MEZ (10 ng/ml)), young fibroblast cultures (GM01379) and two senescent cell cultures (AG01976, AG0989B) were isolated and expression of specific Old genes was determined (Fig. 1). Since the subtracted library that was screened should be enriched for H0-1 genes regulated by IFN-β and IFN-β + MEZ, it was anticipated that the level of expression of many of the Old cDNAs would be reduced or absent in actively proliferating, untreated HO-1 cells. However, since this library was screened with an un-subtracted senescent probe (containing senescent specific, housekeeping and other genes) some of the cDNAs should also be expressed in non-senescent fibroblasts.

Four of the six novel cDNAs, Old-137, Old-139, Old-142 and Old-175, were expressed in both proliferating and senescent fibroblasts. Expression of two novel Old genes, Old-35 and Old-64, were restricted to the senescent fibroblasts and IFN-b and IFN-β + MEZ treated H0-1 cells. Different exposure times revealed that the expression level of Old-35 is higher in senescent fibroblasts than in HO-1 cells treated with IFN-β or IFN-β + MEZ (Fig. 1). Response of Old-35 to Interferons Time-course and dose-response experiments were performed in H0-1 cells to determine the temporal kinetics of Old-35 induction by IFN-β and the concentration of IFN-β capable of inducing Old-35 expression, respectively. Additionally, the effect of IFN-α, IFN-γ and TNF-α on Old-35 expression in H0-1 cells was examined. Old-35 was up-regulated by IFN-β (2,000 units/ml) and IFN-β + MEZ (2,000 units/ml + 10 ng/ml) within 3 hr of treatment (Fig. 2A and D). Since IFN-β induces growth suppression in H0-1 cells at 2,000 units/ml, it was considered important to determine whether up-regulation of Old-35 could occur in the absence of growth suppression. Old-35 expression was induced in H0-1 cells with as little as 1 U/ml of IFN-α, a dose of IFN that is not growth inhibitory, suggesting a direct effect of IFN on expression of this gene in the absence of growth suppression (Fig. 2B). Treatment of H0-1 cells with IFN-α resulted in significant up-regulation of Old-35 in H0-1 cells, whereas this expression was marginally stimulated by IFN-γ and no detectable or consistent induction occurred with TNF-α (Fig. 2C). These experiments document differential regulation of Old-35 expression by different cytokines, with type I Interferons (IFN-α/IFN-β) being the most active cytokines tested in inducing Old-35 expression in H0-1 cells. Expression of Old-35 in various human tissues and during mouse development to determine the tissue-specific expression pattern of Old-35 we examined the expression of this gene using Human Multiple Tissue Northern (MTN) Blots (Clontech) (Fig. 3 A). Old-35 was expressed in all of the tissues tested, including heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. The highest levels of

Old-35 expression were detected in the heart and brain. Since the heart and brain contain a high proportion of non-regenerating, terminally differentiated cells, it is possible that Old-35 may be important in maintaining end stage differentiation in these target organs. Since terminal differentiation of specific tissue cell types occurs during normal development of the embryo, the expression pattern of Old-35 was determined during mouse development. The highest level of Old-35 expression was apparent during the earliest stage of development (8 days) and it steadily declined with time (10 to 16 days) (Fig. 3B). This dilution effect is frequently observed when mRNA expression is localized in a specific organ as the embryo develops, because the ratio of the region of expression to the whole body decreases over time. Since the mouse developmental Northern Blot was probed with human cDNA and the resulting signal was very strong, the homology between human and mouse OLD-35 transcripts must be very high. The EST database search showed very close homology between the mouse and the human cDNA, ~ 90% (Fig. 4). Expression of Old-35 during growth arrest and senescence in IDH4 cell IDH4 cells were produced by transfecting IMR-90, normal human fibroblasts, with a dexamethasone (DEX) inducible mouse mammary tumor virus-driven simian virus 40 T-antigen (Wright et al., 1989). In this model system, prolonged proliferation and the absence of markers of senescence are dependent upon the continued presence of DEX and thus the SV40 T-antigen. (Wright et al., 1989). In DEX-free medium, DNA synthesis declines by ~80% within the first 3 days and reaches a minimum level at day 7. This decline corresponds with a decrease in telomerase activity and T-antigen expression (Holt et al., 1996). Since T-antigen has a long half-life (~3 days) and remains in the cells for about 5-7 days after the removal of DEX, it is possible that the up-regulation of Old-35 by day 7 corresponds with the depletion of T-antigen in these cells (Fig. 5). Further experiments to define relationship between T-antigen expression and Old-35 expression in IDH4 cells are in progress. Old-35 and p21 are coordinately expressed in

quiescent cells since many of the genes involved in terminal differentiation and senescence are predominantly active during the G_1 phase of the cell cycle, we determined whether Old-35 was expressed at this point of the cell cycle. To achieve this objective, human diploid fibroblasts were grown to confluence (a classic way to arrest and synchronize these cells) (*Tseng *et al.*, 1983). After release of the cells from confluence, following a short lag cells re-entered G_1 phase and then the cells traversed though S, G_2, M and back to G_1. In these cells, Old-35 was highly expressed during the confluence period and at G_1 (Fig. 6). Additionally, as more of the cells entered G_1 Old-35 expression increased. After 15hr, Old-35 expression was significantly reduced, but expression increased again when the cells became confluent (20 hr). The expression of p21 (G_1 specific cyclin-dependent kinase inhibitor) coincided with the expression of Old-35 (Fig. 6).

STABILITY OF OLD-35 IN IFN-B TREATED CELLS

The 3' UTR of particular lymphokines, cytokines and proto-oncogenes contain ARE elements that are implicated in regulating mRNA stability (Fig. 7). The presence of four such ARE elements in the 3' UTR of Old-35 suggests that mRNA stability may contribute to differential expression of this gene under varied treatment and growth conditions. Recently, HuR a protein involved in the destabilization of mRNAs containing ARE elements has been purified and identified as a member of the Elav-line gene family (Myer et al., 1997). If the HuR protein can regulate the stability of Old-35 in HO-1 cells, then treatment of cells with cycloheximide, which inhibits protein synthesis, should decrease or eliminate the HuR protein thereby resulting in stabilization of Old-35 mRNA. Cycloheximide treatment of H0-1 cells (Fig. 8A, lane 2) and IFN-b pre-treated H0-1 cells (Fig. 8A, lane 6 and 7) increases the level of Old-35 mRNA indicating that factors responsible for its degradation might have been inhibited. The cycloheximide studies also indicate that induction of

Old-35 expression can occur in the absence of new protein synthesis (Fig. 8A, lanes 3, 4 and 5). However, since Old-35 RNA production in H0-1 cells occurs within 3 hr of treatment, and cycloheximide is present for the entire treatment period, it is possible that modifications of existing proteins may occur prior to changes in Old-35 transcription. Control of mRNA levels in a cell are regulated predominantly at two points: transcription and mRNA stability. To determine if IFN-β + MEZ or IFN-β effect Old-35 mRNA stability in H0-1 cells, the half-life of the Old-35 mRNA was determined as previously described (Jiang et al., 1993b) (Fig. 8B). Untreated and IFN-β + MEZ or IFN-β treated HO-1 cells were incubated with the RNA polymerase II inhibitor Actinomycin D (Act D) and the stability of pre-existing mRNA was determined by Northern blotting. This experiment revealed that the half-life of Old-35 mRNA in HO-1 cells treated with IFN-β + MEZ or IFN-β is ~6-8 hr, suggesting that stabilization of this mRNA may contribute tc the elevation of Old-35 levels in treated cells (Fig. 8B and data not shown). However, because of the low level of Old-35 expression in untreated H0-1 cells, it was not possible to accurately determine the half-life of this message in these cells. Whether the observed low levels of Old-35 mRNA in untreated actively proliferating HO-1 cells are the result of a lack of transcriptional activation or mRNA stability still remain to be determined. Nuclear run-on assays, that measure rate of RNA transcription, should reveal whether the promoter is active in HO-1 cells in the absence of IFN-β treatment and the potential contribution of transcriptional activation to elevated Old-35 mRNA following IFN-β and IFN-β + MEZ treatment.

CLONING AND SEQUENCE ANALYSIS OF OLD-35

An initial 600bp fragment of Old-35 was identified and cloned from a differentiation inducer treated subtracted (DISH) HO-1 cDNA library as described in the library screening protocol. This cDNA was cloned in a pBlueScript vector in the opposite orientation 3'-5' (EcoRI-XhoI) as a result of subtraction

hybridization. During the subtraction procedure, cDNAs are excised from the vector by double-digestion with EcoRI and XhoI. Since many cDNA also contain internal EcoRI-XhoI sites, many cDNAs will be cut internally and after the subtraction procedure they will re-ligate in the incorrect direction. Thus the original 600bp fragment of Old-35 contained an internal region of Old-35 cDNA and lacked 3' and 5' flanking sequences. The 5' region of Old-35 was cloned from IFN-β treated HO-1 cells using a recently developed cDNA extension procedure, complete open reading frame cloning (C-ORF), yielding in a single-reaction an ~2kb fragment (Kang and Fisher, unpublished). The 3' region of Old-35 was cloned using the 3' RACE procedure with 3' gene specific nested primers and dT, yielding an ~400bp product. The final sequence of Old-35 is shown in Fig. 9. Although a portion of the 5' may still be missing, the Old-35 cDNA obtained using C-ORF and 3' RACE represents a near full-length clone judging from the Northern blotting data (Fig. 1), in which Old-35 hybridizes with an ~2.4-2.7 kb RNA species. Sequence analysis revealed that the Old-35 cDNA (~2.6kb) contains a less frequently observed polyadenylation site (AUUAAA) (found in only ~10% of cDNAs) (Manley *et al.*, 1988). The putative protein sequence does not exhibit homology to any known genes except to the *Escherichia coli* PNPase (polyribonucleotide phosphorylase) gene of which 30% of the sequence is homologous and 50% displays sequence similarity (Fig. 10).

EXPERIMENTAL DISCUSSION

Controlled cellular proliferation is paramount for sustaining homeostasis in multicellular organisms. The regulation of this dynamic process is of particular relevance in maintaining a balance between cell loss and cell renewal, important factors in development, differentiation and aging. Moreover, abnormalities in cell division are hallmarks of many disease states, including developmental and congenital birth defects, premature aging syndromes and abnormal proliferative states such as cancer. Several genes involved

in cell proliferation control, including the tumor suppressor p53 and the cyclin dependent kinase (cdk) inhibitor p21, display elevated expression in growth suppressive conditions, such as quiescence (Niculescu et al., 1998, Lacombe et al., 1996, Linke et al., 1996), senescence (Irving et al., 1992; Gire and Wynford-Thomas, 1998) and terminal cell differentiation (Jiang et al., 1994b, 1995b; Steinman et al., 1994). Since both terminal differentiation and senescence are characterized by growth arrest, it is possible that similar and overlapping genes and gene expression changes may mediate these processes. To test this hypothesis we have screened a subtracted differentiation inducer treated human HO-1 melanoma library with mRNA derived from senescent human fibroblasts. This approach has resulted in the isolation of a large number of cDNAs, consisting of both known and novel sequences (Table 1), displaying elevated expression in senescent human fibroblasts. Several of the same cDNAs, have also been independently identified from the same subtracted HO-1 library after screening with mRNA isolated from HO-1 cells treated with IFN-b + MEZ that induce irreversible growth arrest and terminal differentiation (Huang et al., 1999). This observation validates our hypothesis and suggests that this novel approach may prove useful in identifying and cloning genes displaying coordinated expression as a function of induction of growth arrest during terminal differentiation and cellular senescence. One such cDNA is the novel gene, Old-35.

Induction of terminal differentiation in human melanoma cells by IFN-β + MEZ frequently results in the induction and up-regulation of genes that also display elevated expression following exposure to IFN-β, referred to as Type I melanoma differentiation associated (*mda*) genes (Jiang and Fisher, 1993). Old-35 represents such a gene, since its expression is elevated in HO-1 cells after treatment with IFN-β and IFN-β + MEZ. Old-35 is also up-regulated during growth arrest and senescence in human fibroblasts, indicating that its expression is not restricted to only programs of

differentiation or to human melanoma cells. Since IFN-β has well-established antiproliferative properties, in both normal and cancer cells (Fisher and Grant, 1985), it is possible that Old-35 may function as a down-stream gene in the IFN-signaling pathway culminating in growth arrest. A number of experiments indicate that Old-35 expression is related to cellular senescence and proliferative quiescence. Analysis of Northern blots from young versus senescent human fibroblasts indicates restricted expression of Old-35 to senescent cells. IDH4 cells, conditionally immortalized by a DEX-inducible SV40 T-antigen, represent an excellent *in vitro* model to study senescence (Wright et al., 1989). The presence of DEX in the growth media allows the IDH4 cells to actively proliferate, while the absence of it causes them to senesce. In these cells, Old-35 expression is only detected after 7 days of growth in media devoid of DEX. This expression also corresponds with the SA- (-GAL staining of IDH4 cells, a well-established senescence marker (Dimri et al., 1995). Old-35 expression also increases when fibroblasts become arrested in G_o by growth and maintenance at confluence. In these contexts, Old-35 could prove useful as a diagnostic marker for cellular senescence, terminal differentiation and growth arrest. High levels of Old-35 expression are also found in the brain and heart, the only human tissues that do not possess active regenerative properties. Judging from the localized expression of Old-35 during development, this gene may contribute to heart and brain development by assisting in the maintenance of terminal differentiation of cells in these organs. Due to the high sequence homology of Old-35 to bacterial polyribonucleotide phosphorylase (PNPase), it is possible that Old-35 protein may exhibit a PNPase enzymatic activity. PNPase is one of the critical components of the *Escherichia coli* RNA degradosome (Blum et al., 1997), which consists of both PNPase and endoribonuclease RNase E. The function of this complex is to control the rate of mRNA degradation. The PNPase possesses two enzymatic activities, 3'-5'processive exoribonuclease

activity and 5'-3' RNA polymerase activity (Blum et al., 1997). Recently, it has been shown that PNPase also has the capacity to bind to a specific double-stranded DNA sequence in a sequence-specific manner (Zhang et al., 1998). Since Old-35 is differentially expressed in cells that undergo growth arrest, it is possible that this gene may play a role in RNA degradation in growth arrested cells. Additionally, since genes containing AUUUA elements (Myer et al., 1997) have been shown to be involved in the global regulation of gene expression it is possible that Old-35 by binding sequence-specific targets, controls growth related gene expression. In this context, Old-35 might display tumor suppressor properties and could be useful for the gene therapy of cancer.

TABLE 1

CLONE DESIGNATION	CLONE IDENTITY
Old-1	Vimentin
Old-2	Human ribosommal protein S3a, v-fos
Old-5	mRNA M phase phosphoprotein
Old-7	RIG-G, Cig49
Old-11	MHC class I lymphocyte antigen
Old-14	Human non-muscle myosin alkaline light chain
Old-18	Human ADP-ribosylation factor 4
Old-19	Human mitochondrial cytochrome oxidation
Old-24	56 kDa IFN inducible
Old-30	Ribosommal protein L5
Old-32*	Novel*
Old-34	IFN-inducible protein
Old-35*	Novel*
Old-38	H.s. small acidic protein
Old-39	Human acidic ribosomal phosphatase
Old-42	Neurofibromatosis type 1

Old-59	Human nuclear receptor hTAK1
Old-60	Mitochondrial DNA
Old-61	Transcription factor I (99%)
Old-64*	Novel*
Old-65	CDC16HS cell 81, 261-68
Old-74	Human ISG 54K gene (IFN-gamma)-cig42
Old-79	Human T-complex polypeptide I gene
Old-80	Vitamin D induced
Old-83*	Novel*
Old-87*	Novel*, Possibly similar to Old-83
Old-107*	Novel*-Human homologue of Cow G-Protein
Old-113	DNA binding protein
Old-115	U1 small nuclear RNP
Old-119	Human HS1 protein
Old-121*	Novel*
Old-137*	Novel*
Old-139*	Novel*

Old-140	Human putative trans. CA150
Old-142*	Novel*
Old-144	MLN70 calcium- binding
Old-165	T-cell cyclophilin
Old-170	Human homologue of rat zinc transporter
Old-175 (5-3)*	Novel*

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Example #1

Background and significance

During terminal differentiation and senescence many genes are differentially expressed. Two processes that control the overall mRNA levels are transcription and mRNA stability. Since both proliferation and differentiation are dynamic processes requiring continuos regulation (Blau, H.M., 1992, Blau et al., 1992, Blau et al, 1985) a thorough knowledge of the molecular mechanisms that regulate gene expression will significantly contribute to our understanding of development, differentiation and malignancy. Gene expression is regulated by two mechanisms: transcriptional mechanisms which determine the rate of mRNA production and equally important but under-studied post-transcriptional mechanisms which determine the overall amount of protein being produced. The experimental data from *Xenopous laevis, Drosophila melanogaster, Caenorhabditis elegans* document the importance of post-transcriptional mechanisms in early patterning of the embryos which directs correct distribution, stability, and translation of inherited maternal transcripts (Seydoux, G., 1996) Additionally, in plants, it has been shown that it is the post-transcriptional regulation and not transcription that directs the differentiation of chloroplast from its protoplast precursor (Deng and Gruissem, 1987). In mammals, posttranscriptional regulation appears to be important in cells responding to environmental stress, proliferation and differentiation (June et al, 1990,Sierra et. al., 1994)

The sequences responsible for post-transcriptional regulation are found in the 3' untranslated regions (3'UTR) of the transcripts. When orthologous genes were compared, large regions were found to exhibit more than 70% conservation over 300-500 million years of evolution, from mammals, birds, amphibians, or fish (Spicher et al., 1998).

Post-transcriptional regulation of mRNA levels is a pivotal control point in gene expression. Early response genes, such as cytokines, lymphokines and proto-oncogenes are regulated by a cis-acting adenylate-uridylate-rich element (ARE) found in the 3' untranslated region (UTR) of the mRNA (Caput *et al.*, 1986; Shaw and Kamen, 1988; Chen and Shyu, 1995; Myer *et al.*, 1997). Currently, three classes of destabilizing elements have been identified: AUUUA-lacking elements and AUUUA-containing elements grouped into those with scattered AUUUA motifs (such as proto-oncogenes) and those with overlapping AUUUA motifs (such as growth factors) (Chen *et al.*, 1995; Myer *et al.*, 1997). A replacement of 3'UTR containing ARE in place of a 3'UTR of a stable message, such as β-globin or luciferase targets this very stable mRNA for rapid degradation (Shaw and Kamen, 1988, Maddireddi et al., 2000). In contrast, the removal of an ARE stabilizes an otherwise labile message (Miller *et al.*, 1984; Lee *et al.*, 1988)

A pool of genes involved in mRNA stability remains very small. However, one of the best studied family of genes in this area is Elav. Elav, which stands for embryonic -lethal abnormal vision, was first identified in *Drosophila melanogaster*. Deletion mutants of the *elav* gene are embryonic lethal because of lack of neuronal differentiation (Robinow and White, 1991). In mammals and in Xenopus, the elav gene family consists of three members that are developmentally regulated and tissue specific (Hel-N1, HuC, HuD,) and one member that is ubiquitously expressed called HuR (Szabo et al, 1991, Good, 1995, Ma et al., 1996,Antic and Keene, 1997). The mechanism by which Elav genes promote the differentiation of neurons is not completely understood, however, it is known that Elav can bind AU rich elements in ther 3'UTRs of selected genes. By selectively stabilizing selected genes, the overall amount of gene expression changes observed during terminal differentiation of neurons is regulated.

To obtain further insights into 3' UTR stabilization, 3' end maturation has been studied in detail in plants and bacteria. It is worth noting that the protein complexes involved in this process in these two completely different organisms are highly conserved. They are composed of endonucleases, exonucleases, helicases and enolases. *E.coli*, which lives in an energy high environment, has two exonucleases involved in the processing of 3'UTRs: RNase II, which has hydrolytic activity and PNPase (polynucleotide phosphorylase) which has phosphorolytic activity (Higgins et al., 1993). Single mutant of either PNPase or RNase II is viable, whereas double mutants die (Donovan and Kushner, 1986). On the other hand, *B.Subtilis*, which lives in the soil-an energy poor environment-exclusively uses PNPase and lacks RNase II. There may be a few different explanations for the presence of two exonucleases in *E.coli*. Firstly, the two exonucleases may have different specificities. This is supported by the fact that a specific degradation of S20 mRNA accumulates in pnp mutants but it fails to accumulate in rnb (Rnase II) mutants (Mackie, 1989). Another explanation could be that PNPase is phosphorolytic and Rnase II is hydrolytic. As phosphorolysis releases nucleotide diphosphates (Gedefroy-Colburn and Grunberg-Manago, 1966), the energy of the phosphate bond is conserved. Differential use of these two enzymes may reflect an adaptation to changing energy conditions (Deutscher and Reuven, 1991). This model is supported by the fact that *B. subtilis*, which normally inhabits low energy environment, uses PNPase exclusively, while *E.coli* predominantly uses Rnase II. Another interesting point worth noting is that PNPase also functions during competence development of *B.subtilis* . Since compentence is a state during which specialization is acquired, competence has been used as a simple model for differentiation. Genetic competence may be defined as a physiological state enabling a bacterial culture to bind and take up high-molecular-weight exogenous DNA

(transformation). The study of competence genes has permitted their classification into two broad categories. Late competence genes are expressed under competence control and specify products required for the binding, uptake, and processing of transforming DNA. Regulatory genes specify products that are needed for the expression of the late genes (Dubnau,1991). PNPase is necessary for the expression of late competence genes. Transformability of pnp mutant is 1-5% of that seen in wild type strains (Luttinger A, et al.,1996)

In plants, PNPase functions during chlororoplast differentiation where it is involved in processing of plastid 3' UTR (example:petD). It is interesting to note that plastid genes also possess AU rich regions in its 3' UTR. Identically to bacteria, plant PNPase has a 3'-5' processive exonuclease activity that exhibits increased specificity for poly(A) and poly(U). (Hayes et al., 1996)

Human teratocarcinoma cells (NT2) can be differentiated into neurons with retinoic acid treatment and thus provides an excellent model to study neuronal differentiation. Recently it has been shown that a member of elav family, Hel-N1, when transfected into NT2 cells, forms neurites, an early sign of differentiation. However it does not cause terminal differentiation (Antic et al., 1999). Since Old-35 encodes PNPase, a 3'-5' exonuclease involved in degradation of mRNA sequences, it is possible that Old-35 can increase the effects of Hel-N1 in NT2 cells and cause them to differentiate.

Determining the half-life of Old-35 mRNA in HO-1 cells

Since Old-35 has an AU rich 3'UTR (Fig.4) we have speculated that its expression may be regulated by post-transcriptional mechanisms. One way to study post-transcriptional processes is to investigate mRNA half-lives. In a mammalian cell culture system this can be achieved by treating cells with Actinomycin D (AD). Since

AD inhibits RNA polymerase II activity, mRNA synthesis is terminated and the mRNA synthesized before AD treatment is allowed to decay. Total RNA is collected at different time points and quantified using Northern analyses. Using this protocol, we have examined the half-lives of Old-35 mRNA in HO-1, confluent HO-1, IFN-βtreated, and IFN-β+MEZ treated HO-1. The half-life of Old-35 in all the treatments did not change and **(Figure 11)** was estmated to be 6 hr. Since there was no difference in half-life between HO-1 and IFN-β treated HO-1 it is assumed that a post-transcriptional mechanism is not responsible for the upregulation of Old-35 mRNA level in IFN-β treated HO-1.

Expression of Old-35 during growth arrest and senescence of IDH4 and AR5 cells

IDH4 cells were produced by transfecting IMR-90, normal human fibroblasts, with a dexamethasone (DEX) inducible mouse mammary tumor virus-driven simian virus 40 T-antigen (Wright *et al.*, 1989). In this model system, prolonged proliferation and the absence of markers of senescence are dependent upon the continued presence of DEX and thus the SV40 T-antigen. (Wright *et al.*, 1989). In DEX-free medium, DNA synthesis declines by ~80% within the first 3 days and reaches a minimum level at day 7. This decline corresponds with a decrease in telomerase activity and T-antigen expression (Holt *et al.*, 1996). Since T-antigen has a long half-life (~3 days) and remains in the cells for about 5-7 days after the removal of DEX, it is possible that the up-regulation of Old-35 by day 7 corresponds with the depletion of T-antigen in these cells (**Figure 12**). However, there is one drawback associated with using IDH4 cells. Since the expression of T-antigen is dependent upon DEX, a shift of IDH4 cells towards senescence is dependent upon a complete depletion of DEX from the media and serum in which the cells are growing. This is normally achieved by charcoal stripping of the

serum. However, since fetal serum contains vast amount of steroids, it becomes a challenge to do so in completion. Thus, the reproducibility of complete DEX depletion is a problem. To overcome this problem, we used another cell line, AR5. AR5 is very similar to IDH4, except the fact that T-antigen is not DEX inducible but it is rather temperature sensitive. AR5 cells are able to grow rapidly at 35°C since they are expressing T-antigen. When shifted to 39°C, T-antigen is degraded and the cells become senescent. Total RNA was collected from AR5 cells grown at 35°C and from AR5 cells shifted to 39°C. Old-35 was expressed one day after the shift and at the later time points as well **(Figure 12)**. To make sure that the cells had reached senescence when shifted to 35°C, we hybridized the Northern blot to the well characterized senescence marker, p21 (CDK inhibitor) **(Figure 12)**. p21 expression increased in AR5 cells shifted to 39°C and showed a pattern similar to Old-35.

The difference between expression of Old-35 in IDH4 and AR5 cells can be accounted for by the differences in T-antigen depletion. Since T-antigen degrades much faster in AR5 cells (temperature sensitive) than in IDH4 cells (half-life 2-3 days), AR5 cells reach a senescent state at much faster rate than DEX depleted IDH4 cells.

Cloning of the second variant (3.8 kb)

Once most of the sequence was known, the cDNA was screened against the BLAST-EST database. In this search we have identified another version of Old-35 (3.8 kb) which is probably the upper band observed on Northern blots. The 3.8 kb EST was sequenced. The sequence analysis revealed differences in 3' UTRs of the 2.6kb and 3.8kb fragments. This may result from different polyadenylation patterns. To make sure that the upper band on the Old-35 Northern Blot represents the 3.8kb fragment, we will use the 3' UTR of the ATCC clone as a probe (Figure 13)

Old-35-GFP localization

Since no antibody for Old-35 is currently available, we decided to test the localization of Old-35 by creating an N-terminal fusion of Old-35 and GFP (Clontech). Old-35 was cloned in frame with GFP without the first ATG and then transfected into HeLa and HO-1 cells with SuperFect reagent (Clontech). The protein was allowed to express for 24hr. As expected for a degradative enzyme, Old-35 localized to the cytoplasm of Hela (Figure 14) and HO-1(data not shown).

Expression of Old-35 during mouse development

Using Human Multiple Tissue Northern (MTN) Blots (Clontech) we determined that Old-35 was expressed in all of the tissues tested with the highest levels in the heart and brain. Since terminal differentiation of specific tissue cell types occurs during normal development of the embryo, the expression pattern of Old-35 was determined during mouse development. The highest level of Old-35 expression was apparent during the earliest stage of development (8 days) and it steadily declined with time (10 to 16 days). To determine spacial expression of Old-35, in situ hybridization expreriments were performed. Murine Old-35 was expressed in the spinal tube and in the arteries. However more expreriments have to performed to correctly determine the expression pattern (Figure 15)

Effect of different interferon-α subtypes on Old-35 expression of Old-35

All subtypes of IFN-α stimulated Old-35 expression. IFN-α H and αI-stimulated Old-35 in the lowest extent. **(Figure 16)**. The above experiments document differential regulation of Old-35 expression by different cytokines, with type I interferons (IFN--α /IFN-β) being the most active cytokines tested in inducing Old-35 expression in H0-1 cells. Moreover, since IFN signaling cascades

include Jak and Stat activation, they may prove to be important intermediates of Old-35 induction and expression.

Old-35 genomic structure

As described above, we have identified two BACs that were 100% homologous to specific regions of Old-35 cDNA. First BAC (RPCI-11, Plate=702,Col=8, Row=C) (Research Genetics) showed 100% homology to the 2207-2365 region of Old-35 cDNA. The sequenced regions flanking the Old-35 sequence were foreign thus it is highly possible that they are introns. The second BAC (CITBI-E1, clone 2505G20) (Research Genetics) showed 100% homology in 235bp-313bp region of Old-35 cDNA. After sequencing of the BACs, it became apparent that the Old-35 gene is distributed among 28 exons (Table 2).The spaces in the intron column signify no data for the intron size. The intron sizes are being determined.

Interestingly, there are at least three pseudogenes of Old-35 in the human genome. The first one is 92% homologous to the Old-35 cDNA and contains a portion of the cDNA (48bp-1387bp). 5' and 3' ends of the cDNA could not be found on this BAC. The second pseudogene is present on the 3rd chromosome as determined by BLAST search at it contains a cDNA fragment from the 49th nucleotide to the end of cDNA. This pseudogene exhibits 92% homology to the Old-35 cDNA. The third pseudogene also contains a cDNA fragment from 49 bp to 2517 bp. The second and third BACs are 90% homologous. In all cases, all of the BACs are highly mutated and intronless parts of the Old-35 cDNA.

TABLE 2

EXON-INTRON STRUCTURE OF OLD-35

exons	exon size	introns	intron size	exons	exon size	introns	intron size
1	174	1	6000	15	36	15	
2	60	2	1100	16	66	16	
3	74	3	1300	17	89	17	
4	105	4	1100	18	53	18	
5	49	5		19	105	19	
6	63	6		20	72	20	
7	47	7		21	63	21	
8	113	8	6600	22	83	22	
9	186	9	800	23	83	23	
10	51	10	600	24	106	24	
11	57	11	3500	25	55	25	
12	96	12	800	26	77	26	
13	102	13		27	45	27	
14	70	14		28	406		

What is claimed is:

1. An isolated nucleic acid molecule encoding an OLD-35 or OLD-64 protein.

2. The isolated nucleic acid molecule of claim 1 wherein the nucleic acid comprises a nucleic acid having a sequence substantially the same as set forth in SEQ. ID. No.39 or 19.

3. An isolated nucleic acid molecule encoding an OLD-137, OLD-139, OLD-142, or OLD-175 protein.

4. The isolated nucleic acid molecule of claim 3 wherein the nucleic acid comprises a nucleic acid having a sequence substantially the same as set forth in SEQ. ID. Nos.31, 32, 34 or 38.

5. An isolated nucleic acid molecule of claim 1, or 3, wherein the nucleic acid is DNA, genomic DNA, cDNA, synthetic DNA, or RNA.

6. A nucleic acid molecule comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of the nucleic acid molecule of claim 1, or 3.

7. A nucleic acid molecule of claim 6 wherein the nucleic acid is DNA, genomic DNA, cDNA, synthetic DNA or RNA.

8. An antisense nucleic acid molecule comprising a sequence complementary to the nucleic acid of claim 1 or 3.

9. The antisense nucleic acid molecule of claim 8, capable of inhibiting the expression of the hybridized

gene.

10. An isolated nucleic acid molecule of claim 1, 3, or 8 operatively linked to a promoter of RNA transcription.

11. A vector which comprises the isolated nucleic acid molecule of claim 1, 3 or 8.

12. A host vector system for the production of a protein having the biological activity of OLD-35 or OLD-64 protein which comprises the vector of claim 11 in a suitable host.

13. A host vector system for the production of a protein having the biological activity of OLD-137, OLD-139, OLD-142, OLD-175 protein which comprises the vector of claim 11 in a suitable host.

14. A method of producing a protein having the biological activity of OLD-35, OLD-64 OLD-137, OLD-139, OLD-142, OLD-175 protein which comprises growing the host vector system of claim 12, or 13 under conditions permitting production of the protein and recovering the protein so produced.

15. A purified, OLD-35 protein.

16. A purified, OLD-64 protein.

17. A purified, OLD-137 protein.

18. A purified, OLD-139 protein.

19. A purified, OLD-142 protein.

20. A purified, OLD-175 protein.

21. A protein encoded by the isolated nucleic acid molecule of claim 1 or 3.

22. An antibody or antigen-binding fragment thereof that specifically binds to OLD-35, OLD-64, OLD-137, OLD-139, OLD-142 or OLD-175 protein.

23. A monoclonal antibody of claim 22.

24. A method of inhibiting growth of cancer cells comprising contacting the cancer cells with an amount of purified OLD-35, or OLD-64 protein or a portion thereof effective to inhibit growth of cancer cells.

25. A method for reversing the cancerous phenotype of a cancer cell which comprises introducing a nucleic acid comprising an Old-35 or Old-64 gene or a portion thereof into the cell under conditions permitting the expression of the gene so as to thereby reverse the cancerous phenotype of the cell.

26. A method for reversing the cancerous phenotype of a cancer cell in a subject which comprises introducing a nucleic acid molecule comprising an Old-35 or Old-64 gene or a portion thereof into the subject's cancerous cell under conditions permitting expression of the gene in the subject's cell so as to thereby reverse the cancerous phenotype of the cell.

27. The method according to claim 25 or 26, wherein the nucleic acid molecule comprises a vector.

28. The method according to claim 25 or 26, wherein the Old-35 or Old-64 gene is linked to a regulatory

element such that its expression is under the control of the regulatory element.

29. The method according to claim 26, wherein the regulatory element is a tissue specific regulatory element.

30. The method of claim 25 or 26, wherein the nucleic acid molecule is introduced into the cells by naked DNA technology, adenovirus vector, adeno-associated virus vector, Epstein-Barr virus vector, Herpes virus vector, attenuated HIV vector, retroviral vectors, vaccinia virus vector, liposomes, antibody-coated liposomes, mechanical or electrical means.

31. A method for reversing the cancerous phenotype of a cancer cell which comprises introducing OLD-35 or OLD-64 protein into the cancerous cell so as to thereby reverse the cancerous phenotype of the cell.

32. A method for reversing the cancerous phenotype of a cancer cell in a subject which comprises introducing OLD-35 or OLD-64 protein into the subject's cancerous cell so as to thereby reverse the cancerous phenotype of the cell.

33. The method according to claim 25, 26, 31 or 32, wherein the cancer cell is a breast, cervical, colon, pancreatic, thyroid, skin, brain, prostate, nasopharyngeal, lung, glioblastoma multiforme, lymphoma, leukemia, connective tissue, nervous system cell or basal cell.

34. A pharmaceutical composition which comprises an amount of a nucleic acid molecule comprising Old-35, Old-64 gene or portion thereof effective to reverse the cancerous phenotype of a cancer cell and a

pharmaceutically acceptable carrier.

35. The pharmaceutical composition of claim 34, wherein the nucleic acid molecule comprises a vector.

36. The pharmaceutical composition of claim 35, wherein the vector is an adenovirus vector, adeno-associated virus vector, Epstein-Barr virus vector, Herpes virus vector, attenuated HIV vector, retrovirus vector or vaccinia virus vector.

37. A pharmaceutical composition comprising an amount of OLD-35 or OLD-64 protein effective to reverse the cancerous phenotype of a cancer cell and a pharmaceutically acceptable carrier.

38. The pharmaceutical composition of claim 34 or 36, wherein the cancer cell is a breast, cervical, colon, pancreatic, thyroid, skin, brain, prostate, nasopharyngeal, lung, glioblastoma multiforme, lymphoma, leukemia, connective tissue, nervous system or basal cell.

39. A method of determining whether a cell is senescent comprising measurement of the expression of the Old-35 gene, wherein the expression of the Old-35 gene indicates that the cell is senescent.

40. The method of claim 39, wherein the expression of the Old-35 gene is measured by the expression of Old-35 specific RNA.

41. The method of claim 39, wherein the expression of the Old-35 gene is measured by the expression of the OLD-35 protein.

42. A method of determining whether a cell is terminally differentiated comprising measurement of the expression of the Old-35 gene, wherein the expression of the Old-35 gene indicates that the cell is terminally differentiated.

43. The method of claim 42, wherein the expression of the Old- 35 gene is measured by the expression of Old-35 specific RNA.

44. The method of claim 42, wherein the expression of the Old-35 gene is measured by the expression of OLD-35 protein.

45. A method of determining whether a cell is growth arrested comprising measurement of the expression of the Old-35 gene, wherein the expression of the Old-35 gene indicates that the cell is growth arrested.

46. The method of claim 45, wherein the expression of the Old-35 gene is measured by the expression of old 35 specific RNA.

47. The method of claim 45, wherein the expression of the Old-35 gene is measured by the expression of OLD-35 protein.

48. A method of inhibiting growth of cancer cells comprising contacting the cancer cells with an amount of purified OLD-64 protein effective to inhibit growth of cancer cells.

49. A method of inhibiting growth of cancer cells comprising contacting the cancer cells with an amount of purified OLD-64 protein effective to inhibit growth of cancer cells.

50. A method of determining whether a cell is senescent comprising measurement of the expression of the Old-64 gene, wherein the expression of the Old-64 gene indicates that the cell is senescent.

51. The method of claim 50, wherein the expression of the old-64 gene is measured by the expression of Old-64 specific RNA.

52. The method of claim 50, wherein the expression of the Old-64 gene is measured by the expression of the OLD-64 protein.

53. A method of determining whether a cell is terminally differentiated comprising measurement of the expression of the Old-64 gene, wherein the expression of the Old-64 gene indicates that the cell is terminally differentiated.

54. The method of claim 53, wherein the expression of the Old-64 gene is measured by the expression of Old-64 specific RNA.

55. The method of claim 53, wherein the expression of the Old-64 gene is measured by the expression of the OLD-64 protein.

56. A method of determining whether a cell is growth arrested comprising measurement of the expression of the Old-64 gene, wherein the expression of the Old-64 gene indicates that the cell is growth arrested.

57. The method of claim 56, wherein the expression of the Old- 64 gene is measured by the expression of Old-64 specific RNA.

58. The method of claim 56, wherein the expression of the Old- 64 gene is measured by the expression of the OLD-64 protein.

59. A method of regenerating tissues comprising contacting the tissue with an inhibitor of OLD-35 or OLD-64 protein or a protion thereof at a concentration effective to regenerate said tissues.

60. A method of anti-aging in a cell comprising contacting the cell with an agent for inhibiting expression of Old-35 or Old-64 gene at a concentration effective to reverse the aging process in the cell.

61. A pharmaceutical composition for stimulating cell growth comprising a pharmaceutically acceptable carrier and purified Old-35 or Old-64 suppressant at a concentration effective to stimulate cell growth.

62. A method for screening the presence of interferon alpha or beta of a sample comprising steps of:

(a) contacting the sample with cells under conditions permitting expression of Old-35 or Old-64 gene in the presence of interferon alpha or beta; and

(b) determining the expression of the Old-35 or the Old-64 gene, an increase of expression indicates the presence of interferon alpha or beta.

63. A method for detection of the secretion of interferon alpha or beta comprising steps of:

(a) obtaining an appropriate sample from the subject; and

(b) detecting expression of Old-35 or Old-64 gene, the expression of the Old-35 or the

Old-64 gene indicating the secretion of interferon in a subject.

64. A method for monitoring chemotherapy of a subject comprising steps of:

(a) obtaining an appropriate sample from the subject; and

(b) detecting expression of Old-35 or Old-64 gene, the expression of Old-35 or Old-64 gene indicating that the chemotherapy is effective.

65. A method for diagnosis of the proliferating stage of a tumor from a subject comprising steps of:

(a) obtaining an appropriate sample from the subject; and

(b) detecting expression of the Old-35 or the Old-64 gene, the expression of the Old-35 or the Old-64 gene indicating that the tumor is not at a proliferating stage.

66. A kit for diagnosis of the proliferating stage of a tumor, comprising a nucleic acid molecule capable of specifically hybridizing to the nucleic acid molecule of the Old-35 or the Old-64 gene.

67. A kit for diagnosis of the proliferating stage of a tumor, comprising antibody capable of specifically recognizing OLD-35 or OLD-64 protein.

68. A method for identifying an agent that modulates the expression of the Old-35 or the Old-64 gene, comprising:

(a) contacting a candidate agent with a cell transformed or transfected with a reporter gene under the control of a Old-35 or Old-64

promoter or a regulatory element thereof under conditions and for a time sufficient to allow the candidate agent to directly or indirectly alter expression of the promoter or regulatory element thereof; and

(b) determining the effect of the candidate agent on the level of reporter protein produced by the cell, thereby identifying an agent that modulates expression of Old-35 or 64 gene.

69. A method of identifying compounds that induce proliferation or cancerous phenotype, comprising: exposing cell comprising the promoter of Old-35 or Old-64 to the compound and identifying compounds that suppress the Old-35 or 64 promoter.

70. A method of identifying compounds that induces senescence, or terminal differentiation, comprising: exposing the cell comprising the promoter of Old-35 or Old-64 to the compound and identifying compounds that activate the Old-35 or 64 promoter.

71. A method of identifying genes which are common to the pathway of senescence and terminal differentiation comprising steps of:

(a) obtaining a subtrated library which is enriched for genes expressed in terminal differentiation;

(b) screening the library with senescent probe to identify novel genes which are expressed during senescence and terminal differentiation; and

(c) examining the biological activity of the identified gene to determined whether it is expressed during senescence and terminal differentiation.

72. A method of identifying genes which are common to the pathway of senescence and terminal differentiation comprising steps of:

(a) obtaining a subtracted library which is enriched for genes expressed in senescence;

(b) screening the library with terminal differentiation probe to identify novel genes which are expressed during senescence and terminal differentiation; and

(c) examining the biological activity of the identified gene to determined whether it is expressed during senescence and terminal differentiation.

73. The gene identified by the method of claim 71 or 72.

74. A method of degrading specific RNAs in a cell comprising induction of the expression of Old-35 gene.

74. A method of degrading specific RNAs in a cell comrprising introducing a vector into the cell comprising the Old-35 gene.

75. Expression of Old-35 can be used as diagnostic indicator of cellular senescence, terminal differentiation and/or growth suppression.

(a) can be used to determine if a cell has lost proliferative ability and become senescent.

76. Expression of Old-35 can be used as a marker to identify drugs or small molecules that will induce senescence, e.g., to inhibit cancer cell growth or abnormal proliferative states (such as psoriasis, hemangioblastoma, etc.)

77. Expression of Old-35 can be used to identify drugs or

small molecules that will inhibit senescence, possible uses including stimulating tissue regrowth, repair and regeneration.

78. Expression of Old-35 can be used as a marker to identify drugs or small molecules that will induce terminal cell differentiation, e.g., to inhibit cancer cell growth or abnormal proliferative states (such as psoriasis, hemangioblastoma, etc.).

79. Expression of Old-35 can be used to identify drugs or small molecules that will inhibit terminal differentiation, possible uses including stimulating tissue regrowth, repair and regeneration.

80. Expression of Old-35 can be used as marker for detecting cytokines, specifically type I interferons, in biological samples. Since type I interferon, including leukocyte and fibroblast interferons, which activate gene expression through the well characterized Jak and Stat kinase pathways, this gene can be used to monitor for drugs and small molecules that activate these important pathways.

81. The combination of Old-35 with other interacting proteins can be used to target the differentiation of specific target cells. This can result in the reprogramming of pluripotent stem cells to terminally differentiated end cells.

82. Old-35 can be used to selectively stabilize specific mRNAs possibly containing AU rich 3' UTRs (untranslated regions). This effect can result in the sustained expression of genes potentiating or inhibiting cell growth. It could also result in the stabilizing of cytokine genes resulting in increased biological and immunological activity.

83. Old-35 can be used as part of a methodology to polymerize random NTPs into nucleic acids.

84. Old-35 can be used to induce the degradation of specific mRNAs.

FIG. 1

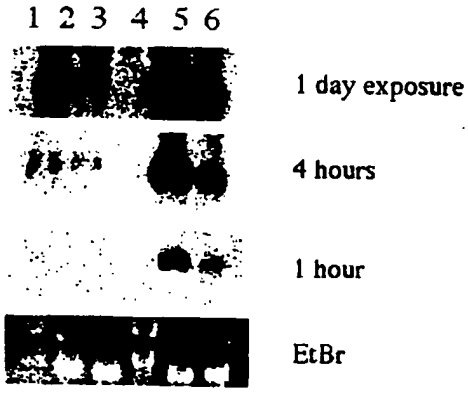


FIG. 2A

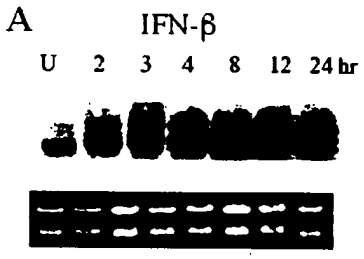
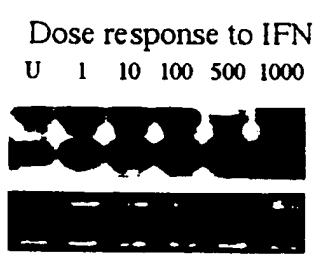


FIG. 2B



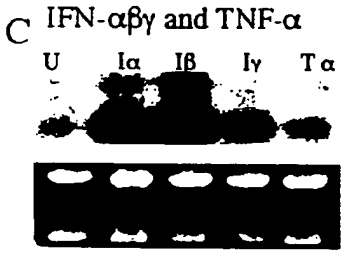


FIG. 2C

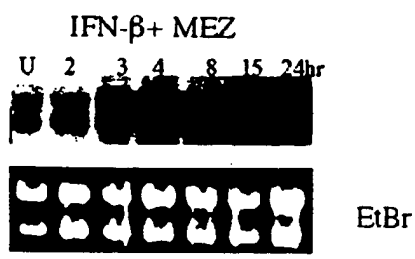


FIG. 2D

FIG. 3A

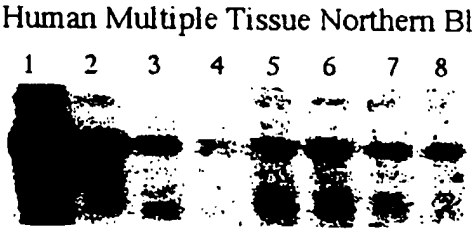

Human Multiple Tissue Northern Blot
1 2 3 4 5 6 7 8

FIG. 3B

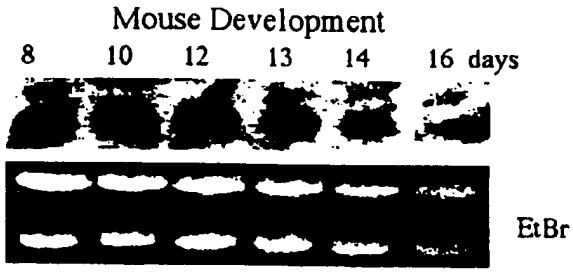

Mouse Development
8 10 12 13 14 16 days
EtBr

FIG. 4A

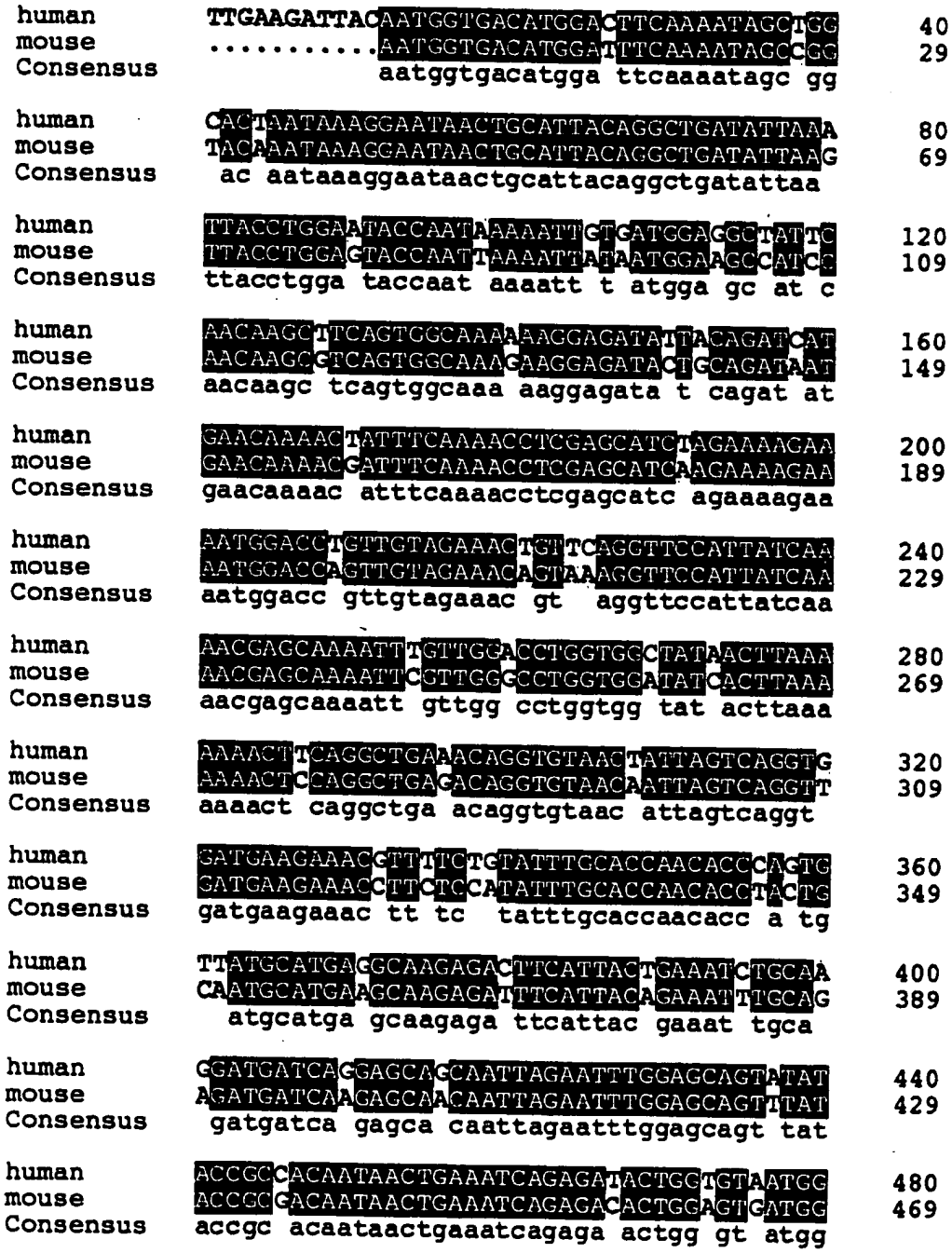

human TTGAAGATTACAATGGTGACATGGACTTCAAAATAGCTGG 40
mouse .....AATGGTGACATGGATTTCAAAATAGCCGG 29
Consensus aatggtgacatgga ttcaaaatagc gg
human CACTAATAAAGGAATAACTGCATTACAGGCTGATATTAAA 80
mouse TACAAATAAAGGAATAACTGCATTACAGGCTGATATTAAG 69
Consensus ac aataaaggaataactgcattacaggctgatattaa
human TTACCTGGAATACCAATAAAAATTGTGATGGAGGCTATTC 120
mouse TTACCTGGAGTACCAATTAAAATTATAATGGAAGCCATCC 109
Consensus ttacctgga taccaat aaaatt t atgga gc at c
human AACAAGCTTCAGTGGCAAAAAAGGAGATATTACAGATCAT 160
mouse AACAAGCGTCAGTGGCAAAGAAGGAGATACTGCAGATAAT 149
Consensus aacaagc tcagtggcaaa aaggagata t cagat at
human GAACAAAAGTATTTCAAAACCTCGAGCATCTAGAAAAGAA 200
mouse GAACAAAACGATTTCAAAACCTCGAGCATCAAGAAAAGAA 189
Consensus gaacaaaac atttcaaaacctcgagcatc agaaaagaa
human AATGGACCTGTTGTAGAAACTGTTCAGGTTCCATTATCAA 240
mouse AATGGACCAGTTGTAGAAACAGTAAAGGTTCCATTATCAA 229
Consensus aatggacc gttgtagaaac gt aggttccattatcaa
human AACGAGCAAAATTTGTTGGACCTGGTGGCTATAACTTAAA 280
mouse AACGAGCAAAATTCGTTGGGCCTGGTGGATATCACTTAAA 269
Consensus aacgagcaaaatt gttgg cctggtgg tat acttaaa
human AAAACTTCAGGCTGAAACAGGTGTAACTATTAGTCAGGTG 320
mouse AAAACTCCAGGCTGAGACAGGTGTAACAATTAGTCAGGTT 309
Consensus aaaact caggctga acaggtgtaac attagtcaggt
human GATGAAGAAACGTTTTCTGTATTTGCACCAACACCCAGTG 360
mouse GATGAAGAAACCTTCTGCATATTTGCACCAACACGTACTG 349
Consensus gatgaagaaac tt tc tatttgcaccaacacc a tg
human TTATGCATGAGGCAAGAGACTTCATTACTGAAATCTGCAA 400
mouse CAATGCATGAAGCAAGAGATTTCATTACAGAAATTTGCAG 389
Consensus atgcatga gcaagaga ttcattac gaaat tgca
human GGATGATCAGGAGCAGCAATTAGAATTTGGAGCAGTATAT 440
mouse AGATGATCAAGAGCAACAATTAGAATTTGGAGCAGTTTAT 429
Consensus gatgatca gagca caattagaatttggagcagt tat
human ACCGCCACAATAACTGAAATCAGAGATACTGCTGTAATGG 480
mouse ACCGCGACAATAACTGAAATCAGAGACACTGGAGTGATGG 469
Consensus accgc acaataactgaaatcagaga actgg gt atgg

FIG. 4B


human TAAAATTATATCCAAATATGACTGCCGTACTGCTTCATAA 520
mouse TAAAACTGTATCCAAACATGACTGCAGTGCTGCTTCATAA 509
Consensus taaaa t tatccaaa atgactgc gt ctgcttcataa
human CACACAACTTGAT.AACGAAAGATTAAACATCCTACTGCC 559
mouse TTCACAACTTGACCAACGAAAGATTAAACATCCCACTGCC 549
Consensus cacaacttga aacgaaagattaaacatcc actgcc
human CTAGGATTAGAAGTTGGCCAAGAAATTCAGGTGAAATACT 599
mouse CTAGGACTAGAGGTTGGCCAAGAAATTCAGGTCAAATACT 589
Consensus ctagga taga gttggccaagaaattcaggt aaatact
human TTGGACGTGACCCAGCCGATGGAAGAATGAGGCTTTCTCG 639
mouse TTGGCCGTGATCCAGCTGATGGAAGAATGAGGCTTTCTCG 629
Consensus ttgg cgtga ccagc gatggaagaatgaggctttctcg
human AAAAGTGCTTC 650
mouse TAAAGTACTTC 640
Consensus aaagt cttc

FIG. 5

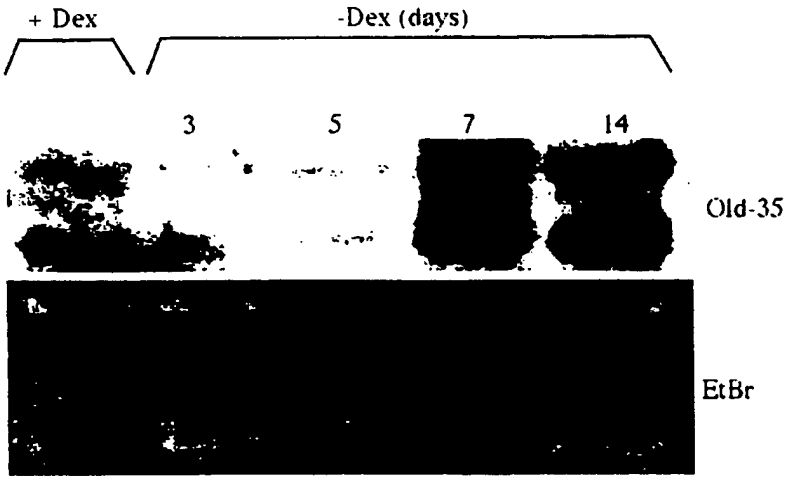


FIG. 6

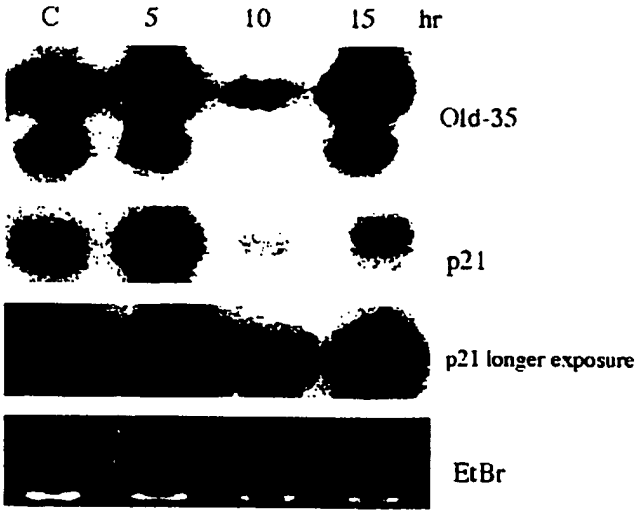


FIG. 7

Hu GM-CSF	UAAUAUUUAUAUAUUUAUAUUUUUAAAAUAUUUAUUUAUUUAUUUAA
Hu IFN-α	UAUUUAUUUAA
Hu Il 2	UAUUUAUUUAAAUAUUUAAAUUUUAUAUUUAAU
Hu TNF	AAUUAAUUUAUUAUUUAUUUAUUAUUUAUUUAUU
C-fos	GUUUUUAAUUUAUUUAUUAAGAUGGAUUCUCAGAUAUUUAUAUUUUUU
	AUUUUAUUUUUUUU
Old-35	AUUUACAUGUGCCAUUUUUUUAAUUCGAGUAACCCAUAUUUGUUUAAUU
	GUAUUUACAUUAUAAAUCAAGAAAUAUUUAUUAUUAAAAGUAAGUC
	AUUUAUACAUCUUAGA

FIG. 8A

Response of Old-35
To IFN-β Treatment
In the Presence of Cyclohexamide

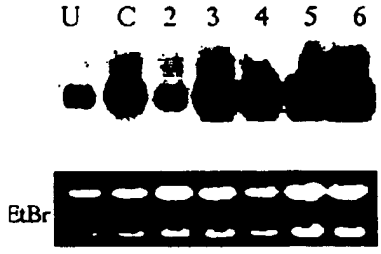


FIG. 8B

Half-life of Old-35 in IFN-β+MEZ
Treated HO-1

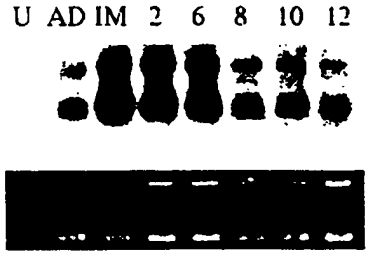


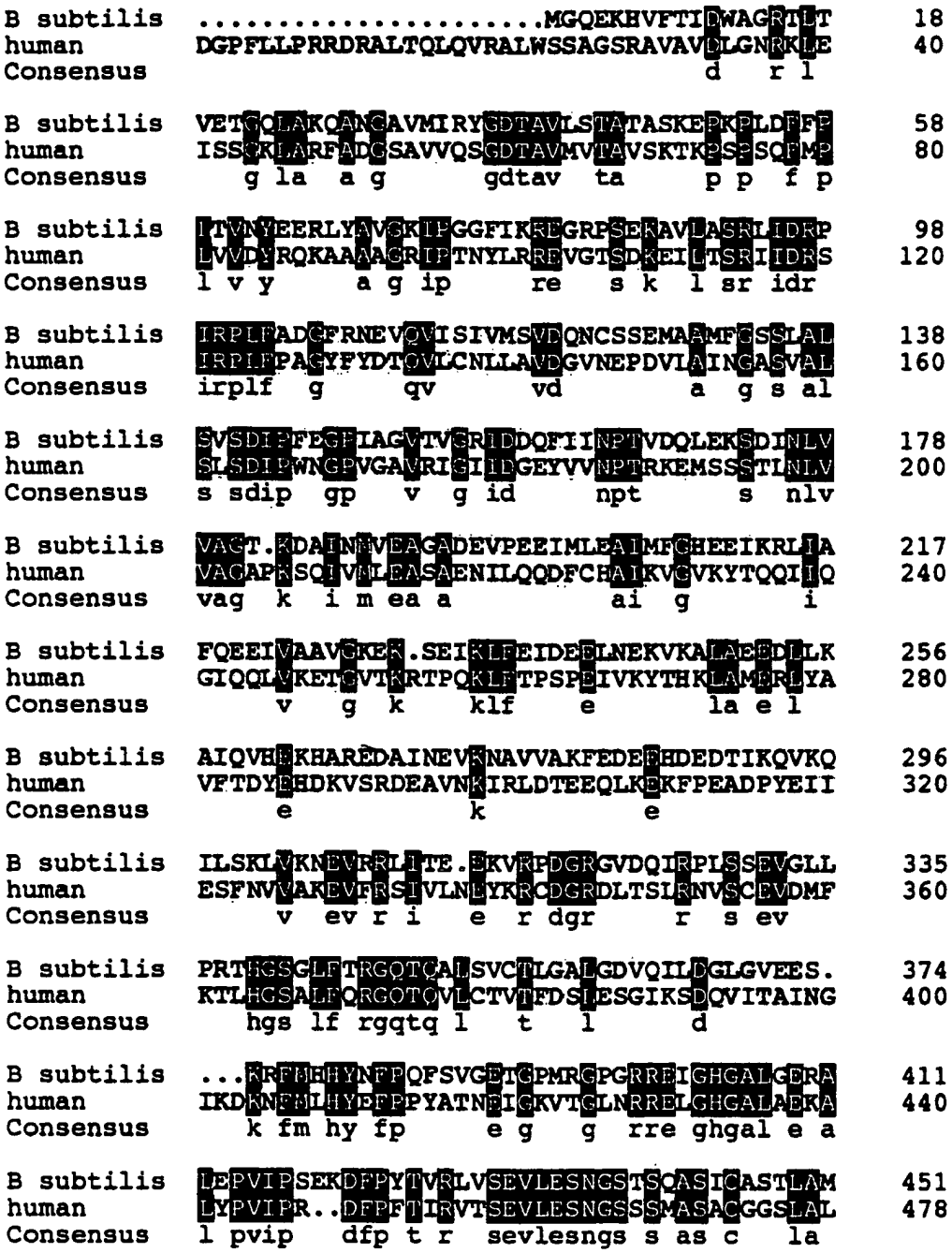
FIG. 9A

GATGGTCCTT TCCTTCTGCC ACGGCGGGAT CGGGCACTCA CCCAGTTGCA
AGTGCGAGCA CTATGGAGTA GCGCAGGGTC TCGAGCTGTG GCCGTGGACT
TAGGCAACAG GAAATTAGAA ATATCTTCTG GAAAGCTGGC CAGATTTGCA
GATGGCTCTG CTGTAGTACA GTCAGGTGAC ACTGCAGTAA TGGTCACAGC
GGTCAGTAAA ACAAAACCTT CCCCTTCCCA GTTTATGCCT TTGGTGGTTG
ACTACAGACA AAAAGCTGCT GCAGCAGGTA GAATTCCCAC AAACTATCTG
AGAAGAGAGG TTGGTACTTC TGATAAAGAA ATTCTAACAA GTCGAATAAT
AGATCGTTCA ATTAGACCGC TCTTTCCAGC TGGCTACTTC TATGATACAC
AGGTTCTGTG TAATCTGTTA GCAGTAGATG GTGTAAATGA GCCTGATGTC
CTAGCAATTA ATGGCGCTTC CGTAGCCCTC TCATTATCAG ATATTCCTTG
GAATGGACCT GTTGGGGCAG TACGAATAGG AATAATTGAT GGAGAATATG
TTGTTAACCC AACAAGAAAA GAAATGTCTT CTAGTACTTT AAATTTAGTG
GTTGCTGGAG CACCTAAAAG TCAGATTGTC ATGTTGGAAG CCTCTGCAGA
GAACATTTTA CAGCAGGACT TTTGCCATGC TATCAAAGTG GGAGTGAAAT
ATACCCAACA AATAATTCAG GGCATTCAGC AGTTGGTAAA AGAAACTGGT
GTTACCAAGA GGACACCTCA GAAGTTATTT ACCCCTTCGC CAGAGATTGT
GAAATATACT CATAAACTTG CTATGGAGAG ACTCTATGCA GTTTTTACAG
ATTACGAGCA TGACAAAGTT TCCAGAGATG AAGCTGTTAA CAAAATAAGA
TTAGATACGG AGGAACAACT AAAAGAAAAA TTTCCAGAAG CCGATCCATA
TGAAATAATA GAATCCTTCA ATGTTGTTGC AAAGGAAGTT TTTAGAAGTA
TTGTTTTGAA TGAATACAAA AGGTGCGATG GTCGGGATTT GACTTCACTT
AGGAATGTAA GTTGTGAGGT AGATATGTTT AAAACCCTTC ATGGATCAGC
ATTATTTCAA AGAGGACAAA CACAGGTGCT TTGTACCGTT ACATTTGATT
CATTAGAATC TGGTATTAAG TCAGATCAAG TTATAACAGC TATAAATGGG
ATAAAAGATA AAAATTTCAT GCTGCACTAC GAGTTTCCTC CTTATGCAAC
TAATGAAATT GGCAAAGTCA CTGGTTTAAA TAGAAGAGAA CTTGGGCATG
GTGCTCTTGC TGAGAAAGCT TTGTATCCTG TTATTCCCAG AGATTTTCCT
TTCACCATAA GAGTTACATC TGAAGTCCTA GAGTCAAATG GGTCATCTTC
TATGGCATCT GCATGTGGCG GAAGTTTAGC ATTAATGGAT TCAGGGGTTC
CAATTTCATC TGCTGTTGCA GGCGTAGCAA TAGGATTGGT CACCAAAACC
GATCCTGAGA AGGGTGAAAT AGAAGATTAT CGTTTGCTGA CAGATATTTT
GGGAATTGAA GATTACAATG GTGACATGGA CTTCAAAATA GCTGGCACTA
ATAAAGGAAT AACTGCATTA CAGGCTGATA TTAAATTACC TGGAATACCA
ATAAAAATTG TGATGGAGGC TATTCAACAA GCTTCAGTGG CAAAAAAGGA
GATATTACAG ATCATGAACA AAACTATTTC AAAACCTCGA GCATCTAGAA
AAGAAAATGG ACCTGTTGTA GAAACTGTTC AGGTTCCATT ATCAAAACGA
GCAAAATTTG TTGGACCTGG TGGCTATAAC TTAAAAAAAC TTCAGGCTGA
AACAGGTGTA ACTATTAGTC AGGTGGATGA AGAAACGTTT TCTGTATTTG
CACCAACACC CAGTGTTATG CATGAGGCAA GAGACTTCAT TACTGAAATC
TGCAAGGATG ATCAGGAGCA GCAATTAGAA TTTGGAGCAG TATATACCGC
CACAATAACT GAAATCAGAG ATACTGGTGT AATGGTAAAA TTATATCCAA
ATATGACTGC GGTACTGCTT CATAACACAC AACTTGATAA CGAAAGATTA
AACATCCTAC TGCCCTAGGA TTAGAAGTTG GCCAAGAAAT TCAGGTGAAA
TACTTTGGAC GTGACCCAGC CGATGGAAGA ATGAGGCTTT CTCGAAAAGT
GCTTCAGTCG CCAGCTACAA CCGTGGTCAG AACTTTGAAT GACAGAAGTA
GTATTGTAAT GGGAGAACCT ATTTCACAGT CATCATCTAA TTCTCAGTGA
TTTTTTTTTT TTAAAGAGAA TTCTAGAATT CTATTTTGTC TAGGGTGATG
TGCTGTAGAG CAACATTTTA GTAGATCTTC CATTGTGTAG ATTTCTATAT
AATATAAATA CATTTTAATT ATTTGTACTA AAATGCTCAT TTACATGTGC
CATTTTTTTA ATTCGAGTAA CCCATATTTG TTTAATTGTA TTTACATTAT
AAATCAAGAA ATATTTATTA TTAAAAGTAA GTCATTTATA CATCTTAGA

FIG. 9B

DGPFLLPRRD RALTQLQVRA LWSSAGSRAV AVDLGNRKLE ISSGKLARFA
DGSAVVQSGD TAVMVTAVSK TKPSPSQFMP LVVDYRQKAA AAGRIPTNYL
RREVGTSDKE ILTSRIIDRS IRPLFPAGYF YDTQVLCNLL AVDGVNEPDV
LAINGASVAL SLSDIPWNGP VGAVRIGIID GEYVVNPTRK EMSSSTLNLV
VAGAPKSQIV MLEASAENIL QQDFCHAIKV GVKYTQQIIQ GIQQLVKETG
VTKRTPQKLF TPSPEIVKYT HKLAMERLYA VFTDYEHDKV SRDEAVNKIR
LDTEEQLKEK FPEADPYEII ESFNVVAKEV FRSIVLNEYK RCDGRDLTSL
RNVSCEVDMF KTLHGSALFQ RGQTQVLCTV TFDSLESGIK SDQVITAING
IKDKNFMLHY EFPPYATNEI GKVTGLNRRE LGHGALAEKA LYPVIPRDFP
FTIRVTSEVL ESNGSSSMAS ACGGSLALMD SGVPISSAVA GVAIGLVTKT
DPEKGEIEDY RLLTDILGIE DYNGDMDFKI AGTNKGITAL QADIKLPGIP
IKIVMEAIQQ ASVAKKEILQ IMNKTISKPR ASRKENGPVV ETVQVPLSKR
AKFVGPGGYN LKKLQAETGV TISQVDEETF SVFAPTPSVM HEARDFITEI
CKDDQEQQLE FGAVYTATIT EIRDTGVMVK LYPNMTAVLL HNTQLDNERL
NILLP·

FIG. 10A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human DGPFLLPRRDRALTQLQVRALWSSAGSRAVAVDLGNRKLE 40
Consensus d r l
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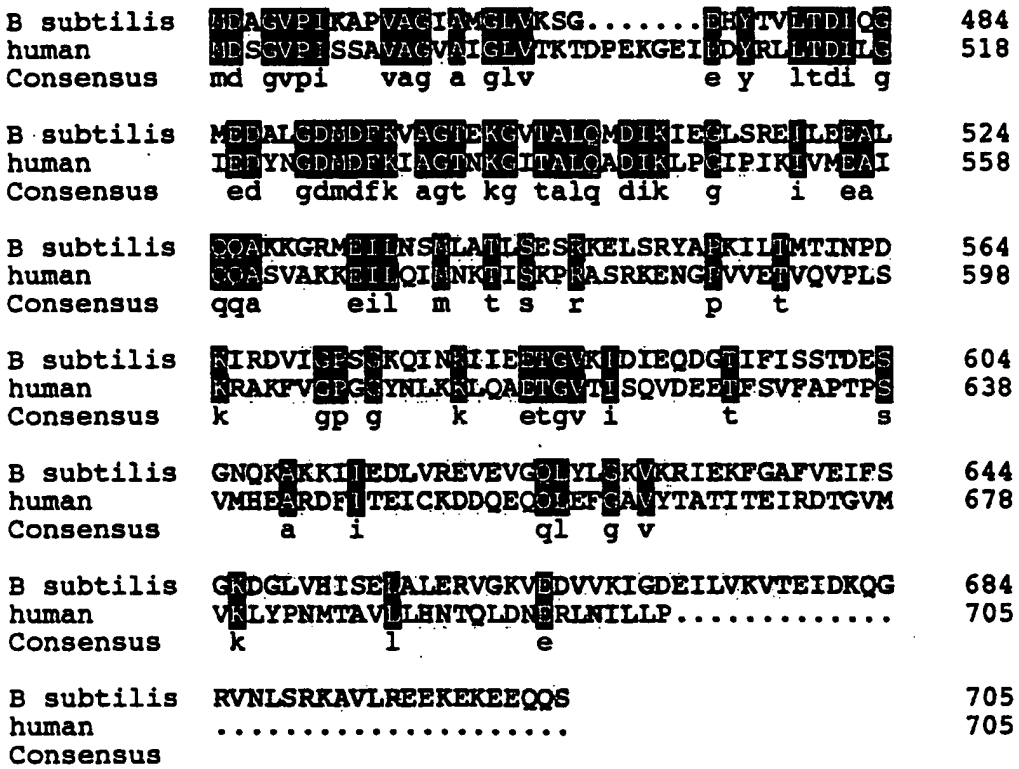
human ISSGKLARFADGSAVVQSGDTAVMVTAVSKTKPSPSQFMP 80
Consensus g la a g gdtav ta p p f p
B subtilis ITVNYEERLYAVGKIPGGFIKREGRPSEKAVLASRLIDRP 98
human LVVDYRQKAAAAGRIPTNYLRREVGTSDKEILTSRIIDRS 120
Consensus l v y a g ip re s k l sr idr
B subtilis IRPLFADGFRNEVQVISIVMSVDQNCSSEMAAMFGSSLAL 138
human IRPLFPAGYFYDTQVLCNLLAVDGVNEPDVLAINGASVAL 160
Consensus irplf g qv vd a g s al
B subtilis SVSDIPFEGPIAGVTVGRIDDQFIINPTVDQLEKSDINLV 178
human SLSDIPWNGPVGAVRIGIIDGEYVVNPTRKEMSSSTLNLV 200
Consensus s sdip gp v g id npt s nlv
B subtilis VAGT.KDAINEVEAGADEVPEEIMLEAIMFGHEEIKRLIA 217
human VAGAPKSQIVTLEASAENILQQDFCHAIKVGVKYTQQIIQ 240
Consensus vag k i m ea a ai g i
B subtilis FQEEIVAAVGKER.SEIKLPEIDEELNEKVKALAEEDLLK 256
human GIQQLVKETGVTKRTPQKLFTPSPEIVKYTHKLAMERLYA 280
Consensus v g k klf e la e l
B subtilis AIQVHEKHARKDAINEVKNAVVAKFEDEFHDEDTIKQVKQ 296
human VFTDYEHDKVSRDEAVNKIRLDTEEQLKEKFPEADPYEII 320
Consensus e k e
B subtilis ILSKLVKNEVRRLITE.EKVRPDGRGVDQIRPLSSEVGLL 335
human ESFNVVAKEVFRSIVLNLYKRCDGRDLTSLRNVSCEVDMF 360
Consensus v ev r i e r dgr r s ev
B subtilis PRTHGSGLFTRGQTQALSVCTLGALGDVQILDGLGVEES. 374
human KTLHGSALLQRGQTQVLCTVTFDSLESGIKSDQVITAING 400
Consensus hgs lf rgqtq l t l d
B subtilis ...KRFHHYNFPQFSVGETGPMRGPGRREIGHGALGERA 411
human IKDKNFNLHYPFPPYATNEIGKVTGLNRRELGHGALAEKA 440
Consensus k fm hy fp e g g rre ghgal e a
B subtilis LEPVIPSEKDFPYTVRLVSEVLESNGSTSQASICASTLAM 451
human LYPVIPR..DFPFTIRVTSEVLESNGSSSMASACGGSLAL 478
Consensus l pvip dfp t r sevlesngs s as c la

FIG. 10B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human
Consensus
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Consensus

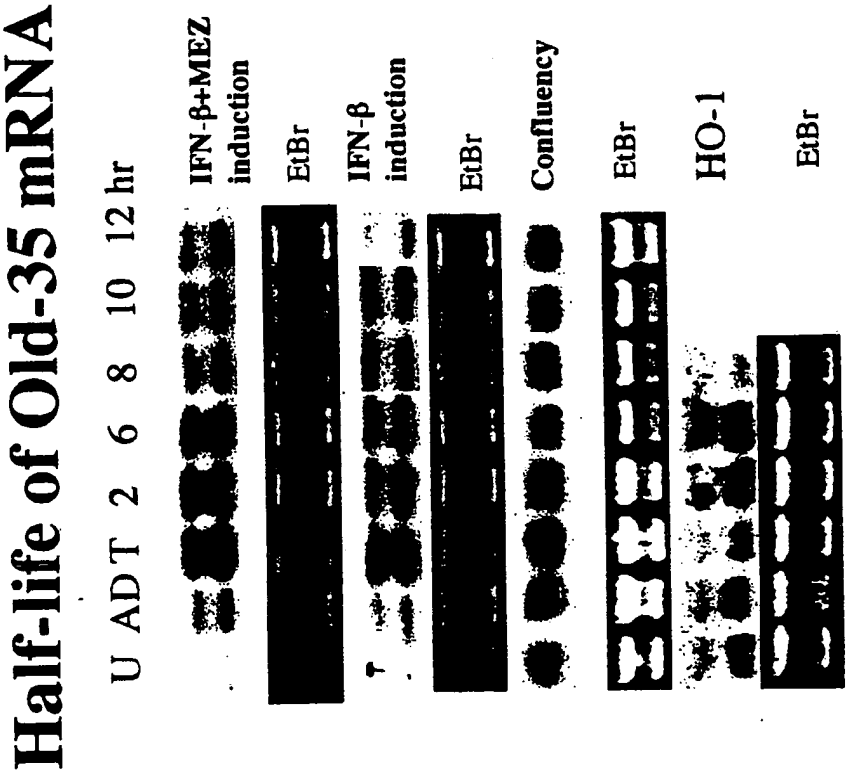

Half-life of Old-35 mRNA
U ADT 2 6 8 10 12 hr
IFN-β+MEZ induction
EtBr
IFN-β induction
EtBr
Confluency
EtBr
HO-1
EtBr

FIGURE 11

FIGURE 12

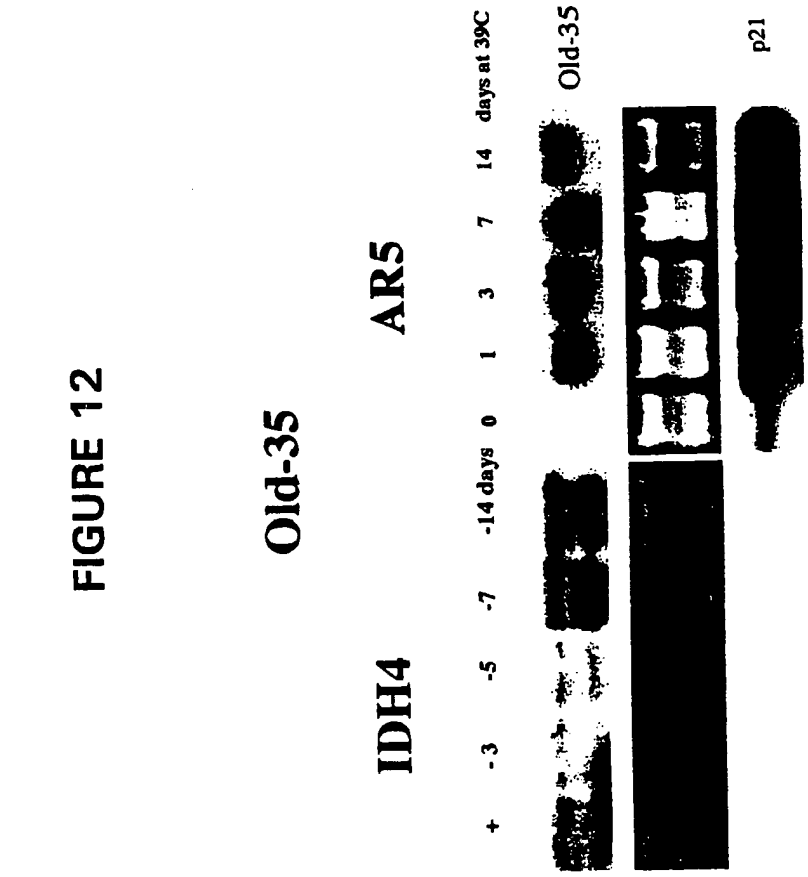
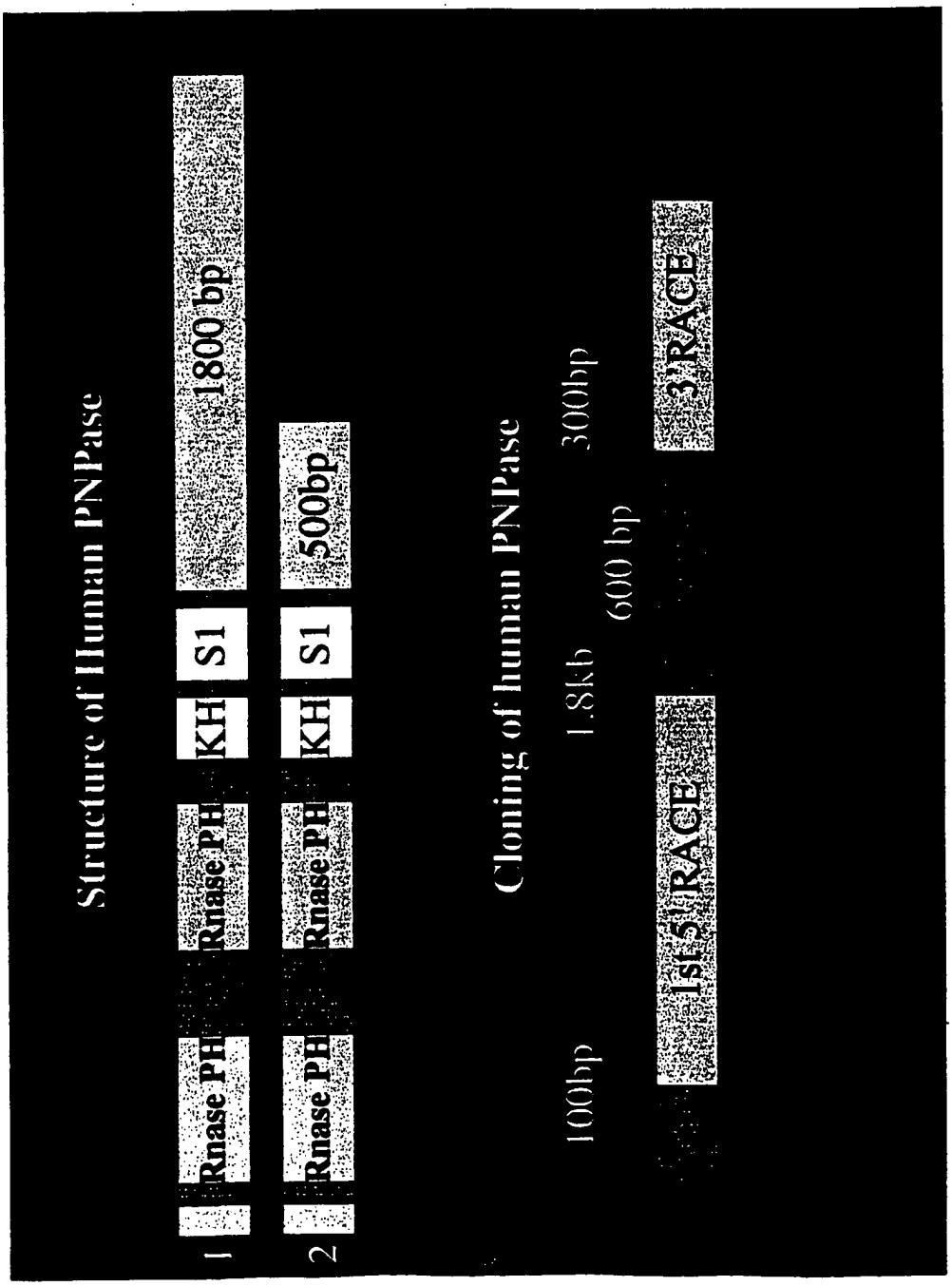


FIGURE 13


Structure of Human PNPase
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Rnase PH
Rnase PH
KH
S1
1800 bp
2
Rnase PH
Rnase PH
KH
S1
500bp
Cloning of human PNPase
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1.8kb
300bp
600 bp
1st 5'RACE
3'RACE

FIGURE 14

The effect of subtypes of IFN- α on Old-35 expression

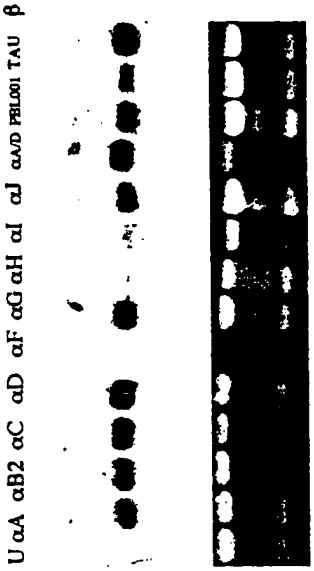
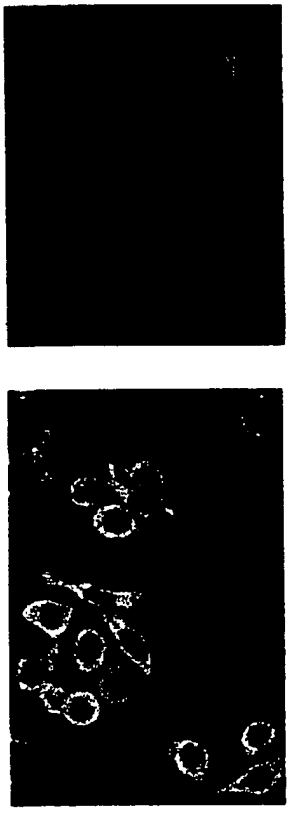


FIGURE 15

Old-35 is expressed in the spinal column and the genital area

FIGURE 16

Localization of Old-35 In HeLa cells



GFP
100X

GFP-hPNPase
40X

SEQUENCE LISTING

<110> Fisher, Paul B.

<120> Genes Displaying Enhanced Expression During Cellular Senescence and Terminal Cell Differentiation and Uses Thereof

<130> 0575/56765

<140> WIPO ST. 10/C
<141> 1999-02-03

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<212> DNA
<213> Homo sapien

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<211> 713
<212> DNA
<213> Homo sapien

<400> 21
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<210> 22
<211> 480
<212> DNA
<213> Homo sapien

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<210> 23
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<212> DNA
<213> Homo sapien

<400> 23
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aaaaaaaaaa aaaaaaaa 198

<210> 24
<211> 414
<212> DNA
<213> Homo sapien

<400> 24
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<210> 25
<211> 367
<212> DNA
<213> Homo sapien

<400> 25
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<210> 26
<211> 432
<212> DNA
<213> Homo sapien

<400> 26
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<211> 398
<212> DNA
<213> Homo sapien

<400> 27
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atcccaanac atcttactga tgcttacttc aagatgaa 398

<210> 28
<211> 232
<212> DNA
<213> Homo sapien

<400> 28
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agagaatagt tttggagggg agaagtggga caaaaaagat gcagttttcc tttgtattgg 180
gaaatgtgaa aataaaattg tcaactcttt caaaaaaaaa aaaaaaaaaa aa 232

<210> 29
<211> 539
<212> DNA
<213> Homo sapien

<400> 29
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gtttctcgtg ctttgtgatc tgttcagtgt cactctgtac cctcaacata tatcccttgt 480
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<210> 30
<211> 568
<212> DNA
<213> Homo sapien

<400> 30
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<210> 31
<211> 315
<212> DNA
<213> Homo sapien

<220>
<223> Human sapien

<400> 31
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<210> 32
<211> 458
<212> DNA
<213> Homo sapien

<400> 32

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tgacntanga atcantaaaa ngatngtttt actctgcaaa aaaaaaaaaa aacncggggg 420
ggggcccggc cccaatttcc ccttntgggg gggggttt 458

<210> 33
<211> 470
<212> DNA
<213> Homo sapien

<400> 33
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gatgtttgaa tgttcagttt atgtatttga actacaataa accaaccctt tttatataaa 420
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<210> 34
<211> 261
<212> DNA
<213> Homo sapien

<400> 34
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aaatctagca gtcatttctg atttcattat attctccccc tcttcctgct aaaaagacaa 240
aaaacaaaaa aaaaaaaaaa a 261

<210> 35
<211> 309
<212> DNA
<213> Homo sapien

<400> 35
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aaaaaaaaa 309

<210> 36
<211> 243
<212> DNA
<213> Homo sapien

<400> 36
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aaa 243

<210> 37
<211> 650
<212> DNA
<213> Homo sapien

<400> 37
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catgataaaa aattaggggg gataaataaa aatggttact ggaatttcaa 650

<210> 38
<211> 687
<212> DNA
<213> Homo sapien

<400> 38
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<210> 39
<211> 2549
<212> DNA
<213> Homo sapien

<400> 39
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<210> 40
<211> 649
<212> DNA
<213> Homo sapien

<400> 40
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<210> 41
<211> 638
<212> DNA
<213> mouse

<400> 41
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<210> 42
<211> 705
<212> PRT
<213> Homo sapien

<400> 42
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Asp Leu Gly Asn Arg Lys Leu Glu Ile Ser Ser Gly Lys Leu Ala Arg
        35                  40                  45

Phe Ala Asp Gly Ser Ala Val Val Gln Ser Gly Asp Thr Ala Val Met
    50                  55                  60

Val Thr Ala Val Ser Lys Thr Lys Pro Ser Pro Ser Gln Phe Met Pro
65                  70                  75                  80

Leu Val Val Asp Tyr Arg Gln Lys Ala Ala Ala Ala Gly Arg Ile Pro
                85                  90                  95

Thr Asn Tyr Leu Arg Arg Glu Val Gly Thr Ser Asp Lys Glu Ile Leu
            100                 105                 110

Thr Ser Arg Ile Ile Asp Arg Ser Ile Arg Pro Leu Phe Pro Ala Gly
        115                 120                 125

Tyr Phe Tyr Asp Thr Gln Val Leu Cys Asn Leu Leu Ala Val Asp Gly
    130                 135                 140

Val Asn Glu Pro Asp Val Leu Ala Ile Asn Gly Ala Ser Val Ala Leu
145                 150                 155                 160

Ser Leu Ser Asp Ile Pro Trp Asn Gly Pro Val Gly Ala Val Arg Ile
                165                 170                 175

Gly Ile Ile Asp Gly Glu Tyr Val Val Asn Pro Thr Arg Lys Glu Met
            180                 185                 190

Ser Ser Ser Thr Leu Asn Leu Val Val Ala Gly Ala Pro Lys Ser Gln
        195                 200                 205

Ile Val Met Leu Glu Ala Ser Ala Glu Asn Ile Leu Gln Gln Asp Phe
    210                 215                 220

Cys His Ala Ile Lys Val Gly Val Lys Tyr Thr Gln Gln Ile Ile Gln
225                 230                 235                 240

Gly Ile Gln Gln Leu Val Lys Glu Thr Gly Val Thr Lys Arg Thr Pro
                245                 250                 255

Gln Lys Leu Phe Thr Pro Ser Pro Glu Ile Val Lys Tyr Thr His Lys
            260                 265                 270
```

```
Leu Ala Met Glu Arg Leu Tyr Ala Val Phe Thr Asp Tyr Glu His Asp
        275                 280                 285

Lys Val Ser Arg Asp Glu Ala Val Asn Lys Ile Arg Leu Asp Thr Glu
    290                 295                 300

Glu Gln Leu Lys Glu Lys Phe Pro Glu Ala Asp Pro Tyr Glu Ile Ile
305                 310                 315                 320

Glu Ser Phe Asn Val Val Ala Lys Glu Val Phe Arg Ser Ile Val Leu
                325                 330                 335

Asn Glu Tyr Lys Arg Cys Asp Gly Arg Asp Leu Thr Ser Leu Arg Asn
            340                 345                 350

Val Ser Cys Glu Val Asp Met Phe Lys Thr Leu His Gly Ser Ala Leu
        355                 360                 365

Phe Gln Arg Gly Gln Thr Gln Val Leu Cys Thr Val Thr Phe Asp Ser
    370                 375                 380

Leu Glu Ser Gly Ile Lys Ser Asp Gln Val Ile Thr Ala Ile Asn Gly
385                 390                 395                 400

Ile Lys Asp Lys Asn Phe Met Leu His Tyr Glu Phe Pro Pro Tyr Ala
                405                 410                 415

Thr Asn Glu Ile Gly Lys Val Thr Gly Leu Asn Arg Arg Glu Leu Gly
            420                 425                 430

His Gly Ala Leu Ala Glu Lys Ala Leu Tyr Pro Val Ile Pro Arg Asp
        435                 440                 445

Phe Pro Phe Thr Ile Arg Val Thr Ser Glu Val Leu Glu Ser Asn Gly
    450                 455                 460

Ser Ser Ser Met Ala Ser Ala Cys Gly Gly Ser Leu Ala Leu Met Asp
465                 470                 475                 480

Ser Gly Val Pro Ile Ser Ser Ala Val Ala Gly Val Ala Ile Gly Leu
                485                 490                 495

Val Thr Lys Thr Asp Pro Glu Lys Gly Glu Ile Glu Asp Tyr Arg Leu
            500                 505                 510

Leu Thr Asp Ile Leu Gly Ile Glu Asp Tyr Asn Gly Asp Met Asp Phe
        515                 520                 525
```

```
Lys Ile Ala Gly Thr Asn Lys Gly Ile Thr Ala Leu Gln Ala Asp Ile
    530                 535                 540

Lys Leu Pro Gly Ile Pro Ile Lys Ile Val Met Glu Ala Ile Gln Gln
545                 550                 555                 560

Ala Ser Val Ala Lys Lys Glu Ile Leu Gln Ile Met Asn Lys Thr Ile
                565                 570                 575

Ser Lys Pro Arg Ala Ser Arg Lys Glu Asn Gly Pro Val Val Glu Thr
            580                 585                 590

Val Gln Val Pro Leu Ser Lys Arg Ala Lys Phe Val Gly Pro Gly Gly
        595                 600                 605

Tyr Asn Leu Lys Lys Leu Gln Ala Glu Thr Gly Val Thr Ile Ser Gln
    610                 615                 620

Val Asp Glu Glu Thr Phe Ser Val Phe Ala Pro Thr Pro Ser Val Met
625                 630                 635                 640

His Glu Ala Arg Asp Phe Ile Thr Glu Ile Cys Lys Asp Asp Gln Glu
                645                 650                 655

Gln Gln Leu Glu Phe Gly Ala Val Tyr Thr Ala Thr Ile Thr Glu Ile
            660                 665                 670

Arg Asp Thr Gly Val Met Val Lys Leu Tyr Pro Asn Met Thr Ala Val
        675                 680                 685

Leu Leu His Asn Thr Gln Leu Asp Asn Glu Arg Leu Asn Ile Leu Leu
    690                 695                 700

Pro
705
```

<210> 43
<211> 665
<212> PRT
<213> Homo sapien

<400> 43

```
Met Gly Gln Glu Lys His Val Phe Thr Ile Asp Trp Ala Gly Arg Thr
  1               5                  10                  15

Leu Thr Leu Thr Val Asn Tyr Glu Glu Arg Leu Tyr Ala Val Gly Lys
```

```
            20                  25                  30

Ile Pro Gly Gly Phe Ile Lys Arg Glu Gly Arg Pro Ser Glu Lys Ala
        35                  40                  45

Val Leu Ala Ser Arg Leu Ile Asp Arg Pro Ile Arg Pro Leu Phe Ala
    50                  55                  60

Asp Gly Phe Arg Asn Glu Val Gln Val Ile Ser Ile Val Met Ser Val
65                  70                  75                  80

Asp Gln Asn Cys Ser Ser Glu Met Ala Ala Met Phe Gly Ser Ser Leu
                85                  90                  95

Ala Leu Ser Val Ser Asp Ile Pro Phe Glu Gly Pro Ile Ala Gly Val
            100                 105                 110

Thr Val Gly Arg Ile Asp Asp Gln Phe Ile Ile Asn Pro Thr Val Asp
        115                 120                 125

Gln Leu Glu Lys Ser Asp Ile Asn Leu Val Val Ala Gly Thr Lys Asp
    130                 135                 140

Ala Ile Asn Met Val Glu Ala Gly Ala Asp Glu Val Pro Glu Glu Ile
145                 150                 155                 160

Met Leu Glu Ala Ile Met Phe Gly His Glu Glu Ile Lys Arg Leu Ile
                165                 170                 175

Ala Phe Gln Glu Glu Ile Val Ala Ala Val Gly Lys Glu Lys Ser Glu
            180                 185                 190

Ile Lys Leu Phe Glu Ile Asp Glu Glu Leu Asn Glu Lys Val Lys Ala
        195                 200                 205

Leu Ala Glu Glu Asp Leu Leu Lys Ala Ile Gln Val His Glu Lys His
    210                 215                 220

Ala Arg Glu Asp Ala Ile Asn Glu Val Lys Asn Ala Val Val Ala Lys
225                 230                 235                 240

Phe Glu Asp Glu Glu His Asp Glu Asp Thr Ile Lys Gln Val Lys Gln
                245                 250                 255

Ile Leu Ser Lys Leu Val Lys Asn Glu Val Arg Arg Leu Ile Thr Glu
            260                 265                 270

Glu Lys Val Arg Pro Asp Gly Arg Gly Val Asp Gln Ile Arg Pro Leu
```

275 280 285

Ser Ser Glu Val Gly Leu Leu Pro Arg Thr His Gly Ser Gly Leu Phe
290 295 300

Thr Arg Gly Gln Thr Gln Ala Leu Ser Val Cys Thr Leu Gly Ala Leu
305 310 315 320

Gly Asp Val Gln Ile Leu Asp Gly Leu Gly Val Glu Glu Ser Lys Arg
325 330 335

Phe Met His His Tyr Asn Phe Pro Gln Phe Ser Val Gly Glu Thr Gly
340 345 350

Pro Met Arg Gly Pro Gly Arg Arg Glu Ile Gly His Gly Ala Leu Gly
355 360 365

Glu Arg Ala Leu Glu Pro Val Ile Pro Ser Glu Lys Asp Phe Pro Tyr
370 375 380

Thr Val Arg Leu Val Ser Glu Val Leu Glu Ser Asn Gly Ser Thr Ser
385 390 395 400

Gln Ala Ser Ile Cys Ala Ser Thr Leu Ala Met Met Asp Ala Gly Val
405 410 415

Pro Ile Lys Ala Pro Val Ala Gly Ile Ala Met Gly Leu Val Lys Ser
420 425 430

Gly Glu His Tyr Thr Val Leu Thr Asp Ile Gln Gly Met Glu Asp Ala
435 440 445

Leu Gly Asp Met Asp Phe Lys Val Ala Gly Thr Glu Lys Gly Val Thr
450 455 460

Ala Leu Gln Met Asp Ile Lys Ile Glu Gly Leu Ser Arg Glu Ile Leu
465 470 475 480

Glu Glu Ala Leu Gln Gln Ala Lys Lys Gly Arg Met Glu Ile Leu Asn
485 490 495

Ser Met Leu Ala Thr Leu Ser Glu Ser Arg Lys Glu Leu Ser Arg Tyr
500 505 510

Ala Pro Lys Ile Leu Thr Met Thr Ile Asn Pro Asp Lys Ile Arg Asp
515 520 525

Val Ile Gly Pro Ser Gly Lys Gln Ile Asn Lys Ile Ile Glu Glu Thr

```
    530                 535                 540

Gly Val Lys Ile Asp Ile Glu Gln Asp Gly Thr Ile Phe Ile Ser Ser
545                 550                 555                 560

Thr Asp Glu Ser Gly Asn Gln Lys Ala Lys Lys Ile Ile Glu Asp Leu
                565                 570                 575

Val Arg Glu Val Glu Val Gly Gln Leu Tyr Leu Gly Lys Val Lys Arg
            580                 585                 590

Ile Glu Lys Phe Gly Ala Phe Val Glu Ile Phe Ser Gly Lys Asp Gly
        595                 600                 605

Leu Val His Ile Ser Glu Leu Ala Leu Glu Arg Val Gly Lys Val Glu
    610                 615                 620

Asp Val Val Lys Ile Gly Asp Glu Ile Leu Val Lys Val Thr Glu Ile
625                 630                 635                 640

Asp Lys Gln Gly Arg Val Asn Leu Ser Arg Lys Ala Val Leu Arg Glu
                645                 650                 655

Glu Lys Glu Lys Glu Glu Gln Gln Ser
            660                 665
```

<210> 44
<211> 704
<212> PRT
<213> Homo sapien

<400> 44

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Asp Gly Pro Phe Leu Leu Pro Arg Arg Asp Arg Ala Leu Thr Gln Leu
  1               5                  10                  15

Gln Val Arg Ala Leu Trp Ser Ser Ala Gly Ser Arg Ala Val Ala Val
             20                  25                  30

Asp Leu Gly Asn Arg Lys Leu Glu Ile Ser Ser Gly Lys Leu Ala Arg
         35                  40                  45

Phe Ala Asp Gly Ser Ala Val Val Gln Ser Gly Asp Thr Ala Val Met
     50                  55                  60

Val Thr Ala Val Ser Lys Thr Lys Pro Ser Pro Ser Gln Phe Met Pro
 65                  70                  75                  80
```

```
Leu Val Val Asp Tyr Arg Gln Lys Ala Ala Ala Ala Gly Arg Ile Pro
                  85                  90                  95

Thr Asn Tyr Leu Arg Arg Glu Val Gly Thr Ser Asp Lys Glu Ile Leu
            100                 105                 110

Thr Ser Arg Ile Ile Asp Arg Ser Ile Arg Pro Leu Phe Pro Ala Gly
        115                 120                 125

Tyr Phe Tyr Asp Thr Gln Val Leu Cys Asn Leu Leu Ala Val Asp Gly
    130                 135                 140

Val Asn Glu Pro Asp Val Leu Ala Ile Asn Gly Ala Ser Val Ala Leu
145                 150                 155                 160

Ser Leu Ser Asp Ile Pro Trp Asn Gly Pro Val Gly Val Arg Ile Gly
                165                 170                 175

Ile Ile Asp Gly Glu Tyr Val Val Asn Pro Thr Arg Lys Glu Met Ser
            180                 185                 190

Ser Ser Thr Leu Asn Leu Val Val Ala Gly Ala Pro Lys Ser Gln Ile
        195                 200                 205

Val Met Leu Glu Ala Ser Ala Glu Asn Ile Leu Gln Gln Asp Phe Cys
    210                 215                 220

His Ala Ile Lys Val Gly Val Lys Tyr Thr Gln Gln Ile Ile Gln Gly
225                 230                 235                 240

Ile Gln Gln Leu Val Lys Glu Thr Gly Val Thr Lys Arg Thr Pro Gln
                245                 250                 255

Lys Leu Phe Thr Pro Ser Pro Glu Ile Val Lys Tyr Thr His Lys Leu
            260                 265                 270

Ala Met Glu Arg Leu Tyr Ala Val Phe Thr Asp Tyr Glu His Asp Lys
        275                 280                 285

Val Ser Arg Asp Glu Ala Val Asn Lys Ile Arg Leu Asp Thr Glu Glu
    290                 295                 300

Gln Leu Lys Glu Lys Phe Pro Glu Ala Asp Pro Tyr Glu Ile Ile Glu
305                 310                 315                 320

Ser Phe Asn Val Val Ala Lys Glu Val Phe Arg Ser Ile Val Leu Asn
                325                 330                 335
```

```
Glu Tyr Lys Arg Cys Asp Gly Arg Asp Leu Thr Ser Leu Arg Asn Val
            340                 345                 350

Ser Cys Glu Val Asp Met Phe Lys Thr Leu His Gly Ser Ala Leu Phe
        355                 360                 365

Gln Arg Gly Gln Thr Gln Val Leu Cys Thr Val Thr Phe Asp Ser Leu
    370                 375                 380

Glu Ser Gly Ile Lys Ser Asp Gln Val Ile Thr Ala Ile Asn Gly Ile
385                 390                 395                 400

Lys Asp Lys Asn Phe Met Leu His Tyr Glu Phe Pro Pro Tyr Ala Thr
                405                 410                 415

Asn Glu Ile Gly Lys Val Thr Gly Leu Asn Arg Arg Glu Leu Gly His
            420                 425                 430

Gly Ala Leu Ala Glu Lys Ala Leu Tyr Pro Val Ile Pro Arg Asp Phe
        435                 440                 445

Pro Phe Thr Ile Arg Val Thr Ser Glu Val Leu Glu Ser Asn Gly Ser
    450                 455                 460

Ser Ser Met Ala Ser Ala Cys Gly Gly Ser Leu Ala Leu Met Asp Ser
465                 470                 475                 480

Gly Val Pro Ile Ser Ser Ala Val Ala Gly Val Ala Ile Gly Leu Val
                485                 490                 495

Thr Lys Thr Asp Pro Glu Lys Gly Glu Ile Glu Asp Tyr Arg Leu Leu
            500                 505                 510

Thr Asp Ile Leu Gly Ile Glu Asp Tyr Asn Gly Asp Met Asp Phe Lys
        515                 520                 525

Ile Ala Gly Thr Asn Lys Gly Ile Thr Ala Leu Gln Ala Asp Ile Lys
    530                 535                 540

Leu Pro Gly Ile Pro Ile Lys Ile Val Met Glu Ala Ile Gln Gln Ala
545                 550                 555                 560

Ser Val Ala Lys Lys Glu Ile Leu Gln Ile Met Asn Lys Thr Ile Ser
                565                 570                 575

Lys Pro Arg Ala Ser Arg Lys Glu Asn Gly Pro Val Val Glu Thr Val
            580                 585                 590
```

Gln Val Pro Leu Ser Lys Arg Ala Lys Phe Val Gly Pro Gly Gly Tyr
595 600 605

Asn Leu Lys Lys Leu Gln Ala Glu Thr Gly Val Thr Ile Ser Gln Val
610 615 620

Asp Glu Glu Thr Phe Ser Val Phe Ala Pro Thr Pro Ser Val Met His
625 630 635 640

Glu Ala Arg Asp Phe Ile Thr Glu Ile Cys Lys Asp Asp Gln Glu Gln
645 650 655

Gln Leu Glu Phe Gly Ala Val Tyr Thr Ala Thr Ile Thr Glu Ile Arg
660 665 670

Asp Thr Gly Val Met Val Lys Leu Tyr Pro Asn Met Thr Ala Val Leu
675 680 685

Leu His Asn Thr Gln Leu Asp Asn Glu Arg Leu Asn Ile Leu Leu Pro
690 695 700

<210> 45
<211> 245
<212> PRT
<213> B subtilis

<400> 45
Asp Arg Leu Gly Leu Ala Ala Gly Gly Asp Thr Ala Val Thr Ala Pro
1 5 10 15

Pro Phe Pro Leu Val Tyr Ala Gly Ile Pro Arg Glu Ser Lys Leu Ser
20 25 30

Arg Ile Asp Arg Ile Arg Pro Leu Phe Gly Gln Val Val Asp Ala Gly
35 40 45

Ser Ala Leu Ser Ser Asp Ile Gly Pro Val Gly Ile Asp Asn Pro Thr
50 55 60

Ser Asn Leu Val Val Ala Gly Lys Ile Met Glu Ala Ala Ala Ile Gly
65 70 75 80

Ile Val Gly Lys Lys Leu Phe Glu Leu Ala Glu Leu Glu Lys Glu Val
85 90 95

```
Glu Val Arg Ile Glu Arg Asp Gly Arg Arg Ser Glu Val His Gly Ser
            100                 105                 110

Leu Phe Arg Gly Gln Thr Gln Leu Thr Leu Asp Lys Phe Met His Tyr
        115                 120                 125

Phe Pro Glu Gly Gly Arg Arg Glu Gly His Gly Ala Leu Glu Ala Leu
    130                 135                 140

Pro Val Ile Pro Asp Phe Pro Thr Arg Ser Glu Val Leu Glu Ser Asn
145                 150                 155                 160

Gly Ser Ser Ala Ser Cys Leu Ala Met Asp Gly Val Pro Ile Val Ala
                165                 170                 175

Gly Ala Gly Leu Val Glu Tyr Leu Thr Asp Ile Gly Glu Asp Gly Asp
            180                 185                 190

Met Asp Phe Lys Ala Gly Thr Lys Gly Thr Ala Leu Gln Asp Ile Lys
        195                 200                 205

Gly Ile Glu Ala Gln Gln Ala Glu Ile Leu Met Thr Ser Arg Pro Thr
    210                 215                 220

Lys Gly Pro Gly Lys Glu Thr Gly Val Ile Thr Ser Ala Ile Gln Leu
225                 230                 235                 240

Gly Val Lys Leu Glu
                245
```

<210> 46
<211> 47
<212> RNA
<213> Homo sapien

<400> 46
uaauauuuau auauuuauau uuuuaaaaua uuuauuuauu uauuuaa 47

<210> 47
<211> 11
<212> RNA
<213> Homo sapien

<400> 47
uauuuauuua a 11

<210> 48
<211> 33
<212> RNA
<213> Homo sapien

<400> 48
uauuuauuua aauauuuaaa uuuuauauuu aau 33

<210> 49
<211> 62
<212> RNA
<213> Homo sapien

<400> 49
guuuuuaauu uauuuauuaa gauggauucu cagauauuua uauuuuuuau uuuauuuuuu 60
uu 62

<210> 50
<211> 111
<212> RNA
<213> Homo sapien

<400> 50
auuuacaugu gccauuuuuu uaauucgagu aacccauauu uguuuaauug uauuuacauu 60
auaaaucaag aaauauuuau uauuaaaagu aagucauuua uacaucuuag a 111